Enspectra Health, Inc.

Study Protocol

Study Title: NONLINEAR IMAGING OF SKIN IN VIVO

NCT #: NCT05410964

Document Date (IRB Approval Date): June 6, 2022

NONLINEAR IMAGING OF SKIN *IN VIVO*

Test device:	*Vio*™		
Clinical study phase:	Feasibility	Date:	01-Jun-2022
Study no.:	CR-30062	Version no.:	A
Sponsor:	Enspectra Health 2495 Hospital Dr, Suite 300, Mountain View, CA 94040 [redacted]		
Investigator:	**Gabriel Sanchez, PhD, CEO** [redacted]		

[redacted]

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Revision History

Version Number	Description of Change	Effective Date
Version 1	Initial release	20 Mar 2017
Version 2	[redacted]	13 Feb 2018
Version 3		13 Aug 2019
Version 4		07 Oct 2021
Version 5-R		02-Mar 2022
Version 6-R		02-May-2022
Version A		01-Jun-2022

List of abbreviations

Abbreviation	Definition
ADE	Adverse device event
AE	Adverse event
CIR	Company-in-residence [redacted]
CRF	Case report form
FDA	Food and Drug Administration (United States)
[redacted]	[redacted]
GCP	Good clinical practices
ICF	Informed consent form
IFU	Instructions for use (User manual)
IRB	Institutional review board
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
PHI	Protected Health Information
SADE	Serious adverse device event
SAE	Serious adverse event
UADE	Unanticipated adverse device effect
US	United States

Study summary	
Title	*Nonlinear imaging of skin* in vivo
Protocol number	[redacted]
Phase	*Feasibility*
Methodology	*Single center, open label, one-arm observational study*
Study duration	*Up to 5 years*
Study center(s)	*Single-Center,* [redacted]
Objectives	*The overall objective of this study is to investigate the potential for the* Vio™ *to display microscopic skin structure in people of different age, sex, race, and skin health.*
Aims	***Aim 1:*** *Quantify imaging performance in various types of skin, which may include estimated* in vivo *resolution and imaging depth. Various types of skin includes skin of different people as well as skin in different locations on the body, and skin with skin disease, including basal cell carcinoma* ***Aim 2:*** *Qualitatively evaluate image quality and interpretability by physicians trained in dermatopathology as a function of skin type.* *Because this study is purely observational, there are no established quantitative endpoints.*
Number of subjects	*Up to 120 subjects*
Inclusion Criteria	*Subjects, ages 2 – 90 years old.* *Subject, or guardian, if applicable, must be willing to provide written informed consent prior to enrollment and agree to comply with protocol requirements. If applicable, child must be willing to provide written assent prior to enrollment and agree to comply with protocol requirements.*

	Subject or guardian, if applicable, must have sufficient mental capacity to understand the Informed Consent and provide clinically relevant and reliable feedback regarding their experience with the device. *Subject and guardian, if applicable, must comply with the protocol requirements.* *Subject or guardian, if applicable, must agree that anonymized personal data will be made available to Study Sponsor and requisite regional and international regulatory bodies.*
Exclusion Criteria	*Any general health condition or systemic disease that may represent, in the opinion of the Principal Investigator, a potential increased risk associated with device use* *Currently infected with a communicable skin infection (e.g., shingles or methicillin-resistant* S. aureus*), which does not include local and minimally pathogenic or non-pathogenic infections distant from the imaging location(s) (e.g., warts, acne)* *Any known allergies to any materials used in the preparation of skin and/or device use* *Has a temporary or permanent electrical implanted medical device*
Study product	Vio™ *system – nonlinear microscopy imaging system; blunt, non-invasive, low-power infrared light, without exogenous dye*
Duration of subject's participation	*One visit up to multiple visits; typically 1 hour per visit, no more than 3 hours per visit. Subjects will be monetarily compensated up to $100 per visit.*

1 Introduction

1.1 Background

Cellular pathology has long provided a powerful means to identify diseases of solid tissues. The emergence of the microscope as the centerpiece of pathology transformed our understanding and diagnosis of cell-based disease, from neoplasia to infarction to infectious disease.[1] In particular, our understanding of cancer has been heavily influenced by surgical pathology since the emergence of anesthesia and aseptic technique in the late 1800s.[2]

Basal cell carcinoma and squamous cell carcinoma, the most common forms of cancer, as well as melanoma occur in the skin and are diagnosed using invasive biopsies paired with cellular pathology. Many other non-malignant skin diseases, such as fungal infection, are also confirmed with cellular pathology. These pathologies occur in the top layers of skin, less than 0.5 mm below the surface. Biopsies for malignancies of the skin are ultimately found to be benign over 70% of the time[3], which means that, each year, patients with ultimately healthy skin experience the risk, inconvenience, and, possibly, anxiety of over 13 million invasive biopsies every year[4]. As such, the ability to visualize skin cellular anatomy noninvasively represents an important unmet medical need.

No clinically-feasible imaging system allows for noninvasive access to skin cellular anatomy. For the past two decades, second harmonic generation and two-photon autofluorescence, collectively called nonlinear imaging, have been used to image skin[5]. These nonlinear imaging systems, however, are too bulky, slow, and expensive for clinical feasibility. Recently, the invention of extreme miniaturization of nonlinear imaging systems has opened up the possibility of bringing this powerful technology to the clinic[6].

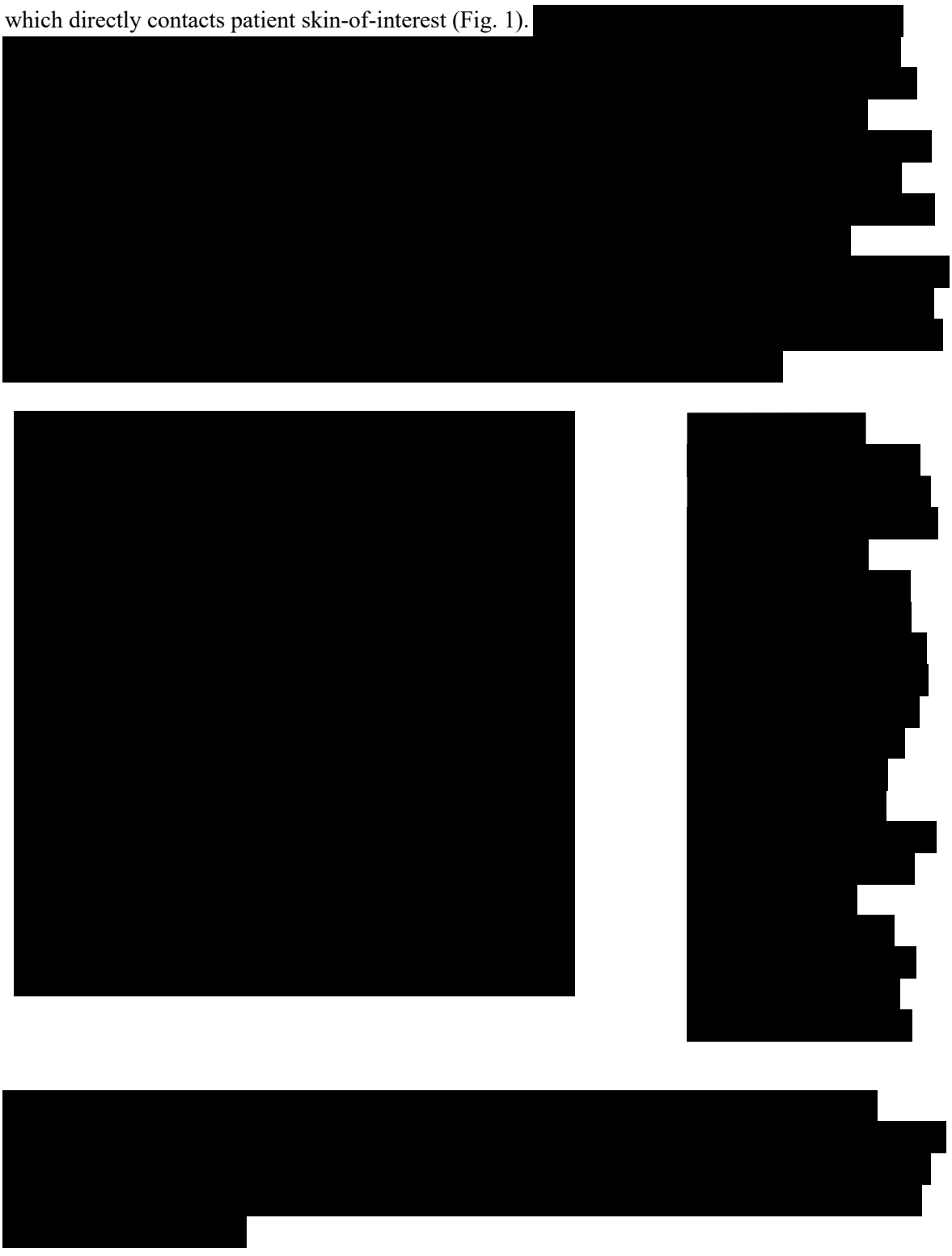
The investigation of a noninvasive, nonlinear microscope to image healthy human skin will allow for the characterization of the imaging performance of this important new technology in a variety of skin types. The ability to visualize cellular architecture of skin without gathering a destructive biopsy may pave the way for rapid, noninvasive surgical pathology in the future both in skin and throughout the body.

1.2 Investigational device: Vio™

Enspectra Health has developed a noninvasive and real-time imaging technique that makes it possible to provide histology-like images of epithelial tissue with subcellular resolution and dye-free color contrast. [redacted] The technique uses the intrinsic signals from second harmonic generation created by collagen and two-photon autofluorescence created by cytoplasmic molecules; thus, imaging is performed without exogenous dyes.

Our system consists of 2 primary components: **(1) an imaging hub**, which contains the near infrared pulsed light source, the control electronics, the optical sensors, and a monitor, which may be optionally placed on a wheeled cart, **(2) a handheld imaging wand**, which is tethered to the hub by fiber optic and electrical cables and held by the user, and includes a cleanable imaging probe,

which directly contacts patient skin-of-interest (Fig. 1). [redacted]



1.3 Clinical data to date

Two-photon fluorescence microscopy has been used to image human skin *in vivo* for nearly two decades[5], although not in a portable format. Spectroscopic data suggest that reduced nicotinamide adenine dinucleotide phosphate [NADPH] is the primary source of two-photon autofluorescence

with a 730 nm excitation source. NADPH is primarily found in cellular cytoplasm outside of the nucleus, resulting in images of cells that reveal cellular shape and size[5] and an absence of signal from the nucleus which may suggest nuclear shape and size as well.

Both two-photon microscopy and second harmonic generation imaging can be performed simultaneously to create a multi-color image with just a single laser light source because the wavelengths of the resulting light from two-photon autofluorescence and second harmonic generation are spectrally separated. When both modalities are combined to image skin, second harmonic generation microscopy provides a view of the connective tissue-rich dermal layer, which is primarily composed of collagen, while two-photon fluorescence microscopy provides of view of cell morphology in the epidermal layer[7]. Nonlinear imaging of skin *in vivo* has been used successfully to identify cellular features characteristic of malignancy by several groups in multiple studies[8–16].

1.4 Risks and benefits

1.4.1 Benefits

There is no direct health benefit to the participant.

The primary benefit of this study comes from the scientific knowledge gained about the capabilities of nonlinear imaging to display clinically-relevant features of cellular structure and architecture noninvasively. The ability to noninvasively inspect skin cellular structure will deepen our understanding of skin physiology, pathology and enable longitudinal follow up in future studies. Such technology may also hasten the diagnosis of malignancy and other skin pathologies by lowering patient burden (pain, risk of scarring, anxiety) compared to invasive biopsies.

1.4.2 Risks

The risks of this protocol are considered to be no more than minimal. The *Vio*^{TM}procedure described in this protocol is similar to a commonly performed clinical procedure: dermoscopy. Like dermoscopy, the *Vio*^{TM}procedure involves non-invasive illumination of skin and magnification of skin structures. Dermoscopy is generally considered to be safe; dermoscopy systems are typically classified by the United States Food and Drug Administration as Class I medical devices, except for Visiomed's microDERM, which, unlike *Vio*^{TM}, involves computer-assisted diagnosis and is considered a Class II medical device. Unlike medical devices, *Vio*^{TM} will not be used to diagnose or treat disease in this protocol, even though images may be taken of skin with disease.

A systematic review found that, among the 433 abstracts that addressed dermoscopy, "no study systematically assessed harms to either patients or the operators from the use of dermascope." Two case reports described possible harm due to dermoscopy: (1) dermoscopy immersion oil contaminated with cedarwood oil was reported to have caused allergic contact dermatitis in one patient, and (2) intensive use of dermoscopy may have caused tonic pupil (Adie's pupil) in a dermatologist[17]. We will limit the risk of allergic contact dermatitis by using an immersion fluid approved to be used in medical settings (e.g., pharmaceutical-grade mineral oil, antibacterial alcohol gel, ultrasound gel). Additionally, the *Vio*^{TM} device comprises materials generally considered biocompatible when used in similar devices for similar applications (*e.g.*, 316L steel,

medical-grade silicone). Concerns have also been raised about patient risk for nosocomial infection when contact dermoscopy is used and the dermascope is not fully cleaned between patients, although it should be noted that dermoscopy is typically used to image intact skin[17]. We will mitigate the risk of infection in this protocol by cleaning the probe between subjects. We may additionally use a single-patient-use, disposable silicone barrier, which may also diminish infection risk. The user applying excessive pressure during use may cause contusions to the skin at the site of imaging. This risk will be mitigated by instructing the user to avoid applying excessive pressure. *Vio™* imaging and risks vary slightly from those of dermoscopy because the light source used is a near infrared laser instead of white light.

Skin: The *Vio™* takes an image of skin tissue by exciting the tissue with laser light, which could introduce the risk of tissue damage if the light power was above a safety threshold. The *Vio™*, however, uses an ultrafast pulsed laser to maintain an overall low average power level[18] so tissue is not damaged, while still providing the high instantaneous intensities needed for nonlinear imaging. Intensity powers are less than 10 kW/μm^2, which are less than powers associated with tissue damage (16 kW/μm^2)[19].

Eyes: Light from the probe tip diverges rapidly, which decreases light power density as a function of distance from the probe tip (Class 1M laser). The Investigator may not choose to investigate the eyelid to remain consistent with earlier data collection in this protocol. Recent testing of the device reveals no substantial risk to the eyes of the patient or user.

Of note, no adverse events have been reported with the use of nonlinear imaging of skin *in vivo* by other groups[8–16].

Relevant risk analyses have been conducted in relation to imaging accessible skin surfaces. Deliberate misuse of the device to image tissues not approved on this protocol may introduce additional risks. These tissues and associated risks include but are not limited to the cervix, gastrointestinal tract, and oral cavity (infection because the non-disposable components have not been designed for the more stringent disinfection that would be required between patients); and interior tissues accessed surgically (infection because the device has not been designed to be used in a surgical sterile environment).

Finally, because the device may be used to image skin with known or suspected skin disease, there is a risk that the *Vio™*, a research-use only device, could improperly influence healthcare decisions or be perceived by the subject to be a part of disease diagnosis. To minimize this risk, images gathered with the *Vio™* paired with PHI will not be shared with the subject's physician or the recruiting physician, if applicable. Any physicians involved in recruitment will be specifically trained that the *Vio™* is for research-use only. The ICF will emphasize to the subject that neither the device nor the study impacts disease diagnosis or treatment.

1.4.3 Risk versus benefits

The minor risks associated with this study are outweighed by the scientific knowledge to be gained about a new imaging technology that may decrease the need to invasively biopsy patients in the future.

2 Investigator(s) and other study personnel

Whenever the term 'Investigator' is noted in the protocol text, it may refer to either the Principal Investigator at the site, or an appropriately qualified, trained, and delegated individual of the investigational site.

The study sponsor, termed 'Sponsor', is identified on the title page of this protocol, and also serves as the Sponsor-Investigator. The Sponsor will select qualified Investigators from within the organization, obtain signed study agreements, and provide the Investigators with the information necessary to conduct the study. Training on the *Vio*^{TM} procedure will occur.

The Principal Investigator must sign the protocol signature sheet before subject recruitment may start at the respective center. Likewise, all protocol amendments/integrated protocols must be signed and dated by the Principal Investigator before coming into effect at the respective center.

A complete list of all participating centers and their Investigators, as well as all required signature documents, will be maintained in the Sponsor-Investigator study file.

All other study personnel not included in this section are identified in a separate personnel list (not part of this clinical study protocol) as appropriate. This list will be updated as needed.

3 Study objectives

The overall objective of this study is to investigate the potential for the *Vio*^{TM} to display microscopic skin structure in people of different age, sex, race, and skin health.

3.1.1 **Aim 1**

Quantify imaging performance in various types of skin, which may include estimated in vivo *resolution and imaging depth. Various types of skin includes skin of different people, skin in different locations on the body, and skin with skin disease, including basal cell carcinoma.*

3.1.2 **Aim 2**

Qualitatively evaluate image quality and interpretability by physicians trained in dermatopathology as a function of skin type.

[redacted]

The safety of device usage will also be evaluated by monitoring adverse events as described in Section 9.

4 Study design

4.1 Overview

This is a single-center, open-label, one-arm observational clinical study. Up to 120 subjects will be enrolled in the study. Each subject will participate in one session for a maximum of 3 hours, but typically

lasting an hour, including the preparation and consent process. Subjects will be monitored for AEs during and immediately following the procedure. Subjects will be requested to self-report any AEs for up to seven days following the procedure. The overall study duration is expected to be approximately 5 years

4.2 Justification for clinical study design

Initial human testing (described in Section 1.3) demonstrates decades of scientific research confirming successful nonlinear imaging of skin *in vivo*. The study described herein will provide observational evidence for the effectiveness of the *Vio*^{TM}, a miniaturized nonlinear microscope, to display features of skin structure as well.

In this study, we plan to gather feasibility data for future studies. This feasibility data will likely inform future clinical investigations of skin disease diagnosis and may assist with sample size calculations.

5 Subject selection and withdrawal

The target patient population is composed of male and female adults and children that meet eligibility criteria described in Sections 5.1.1 through 5.1.5.

5.1 Inclusion criteria

- Subjects, ages 2 – 90 years old.
- Subject or guardian, if applicable, must be willing to provide written informed consent prior to enrollment and agree to comply with protocol requirements. If applicable, child must be willing to provide written assent prior to enrollment and agree to comply with protocol requirements.
- Subject or guardian, if applicable, must have sufficient mental capacity to understand the Informed Consent and provide clinically relevant and reliable feedback regarding their experience with the device.
- Subject and guardian, if applicable, must comply with the protocol requirements.
- Subject or guardian, if applicable, must agree that anonymized personal data will be made available to Study Sponsor and requisite regional and international regulatory bodies.

5.2 Exclusion criteria

- Subjects that meet any of the following exclusion criteria will not participate in the study.
- Any general health condition or systemic disease that may represent, in the opinion of the Principal Investigator, a potential increased risk associated with device use.
- Currently infected with a communicable skin infection that may represent, in the opinion of the Investigator, a potential increased risk to other subjects or users of the device (e.g., shingles or methicillin-resistant *S. aureus*), which does not likely include local and non-pathogenic or minimally pathogenic infections distant from the imaging location(s) (e.g., warts, acne)
- Any known allergies to any materials used in the preparation of skin and/or device use.
- Has a temporary or permanent electrical implanted medical device

5.3 Subject recruitment and screening

Subjects will be recruited for the study using advertisements communicated with flyers, pamphlets, classified ads in local newspapers, electronic posts to sites such as Craigslist.com or SUPost.com, emails to listservs, word-of-mouth, and/or physician referral/recruitment. If a subject is recruited by physician, with permission of the possible subject, the physician may optionally mark a region of interest of skin to be investigated to assist the other study staff (e.g., with a small bandage or a small mark with skin marker). This marking process will likely occur before informed consent for the study has been received because the full explanation of the study will be presented in a separate space without the referring physician present. The marking is temporary; informed consent for the study will be conducted within an hour of marking placement.

After the subject provides written informed consent, the subject will be screened by the Investigator or designee using the protocol defined inclusion and exclusion criteria to determine study eligibility.

5.4 Early withdrawal of subjects

Subjects may be withdrawn from the study prior to the expected completion of that subject for the following reasons:

- Any reason that, in the opinion of the Investigator, poses a safety hazard to the subject
- Failure of subject to adhere to protocol requirements, including but not limited to
  - Inability to stay awake in order to follow instructions
  - Inability to stay still for high quality images
  - Not following Investigator's or designee's instructions
- Subject consent withdrawal

If imaging has already begun when the subject is withdrawn, the laser will be shuttered or turned off and imaging will cease. Abrupt termination of the study will not affect subject safety. Any data collected until withdrawal may still be included in study results.

6 Inclusion of vulnerable populations

6.1 Children

6.1.1 **Reason(s) for choosing this subject population**

Children have been selected as a vulnerable population that may be included in this study because children as a group may particularly benefit from the results of the study, although individual child subjects will not benefit.

The noninvasive technology presented in this protocol may one day reduce the need for invasive skin biopsies. While a biopsy of the skin with local anesthetic is typically considered a minor surgical procedure for adults, for non-assenting or frightened children, skin biopsies may require the use of general anesthesia, sedation[20], or even physical restraint. Participation of children in early feasibility studies such as the one detailed in this protocol will better ensure that noninvasive imaging technology is applicable to this important population.

Although children will be invited to participate, there are no required enrollment targets for children, and the study may be successfully completed without the participation of children.

6.1.2 Level of risk

The risk of this protocol is no more than minimal and is the same for the population of children as for the larger eligible population.

6.1.3 Consent, assent, and coercion

Children may not have the cognitive ability to fully understand the study, risks of the study, and that participation is voluntary, which is why they are considered to be a vulnerable population.

6.1.4 Consenting plan and special protections

Children shall be eligible for this protocol. The group benefits to children of this human research, with special protections, outweigh children's diminished consenting capacity, especially since the risks of the study are no more than minimal.

In order for a child to participate, a parent or legal guardian must provide permission and the child must assent. The full study will be described to the parent or guardian using the consent form. Assent shall be appropriate for the child's age: (1) children under age 7 will be asked to verbally assent, if possible and (2) children aged 7-17 will read (or be read) the child's assent form, and will sign the assent form. Every child will have the option to read the entire consent form if he or she wishes and is able. All children will have the study explained to them at their understanding level by the Investigator. [redacted]

[redacted]

[redacted]

[redacted]

7 Study procedures

Study procedures described below are also shown in schematic form in Figure 3.

7.1 Informed consent, screening, & baseline

7.1.1 **Consent**

The subject will come to the investigation space where the *Vio*^{TM} is set up and may be escorted by study staff between referring physician and the research space, which may be in the same building. The Investigator(s) will fully explain the nature and purpose of the research and procedures, answer questions from the subject, and will obtain the proper signatures of subject and/or guardian on the informed consent form. If, in the Investigator's judgment, the subject or guardian cannot fully understand the procedure or communicate understanding, consent or permission, if applicable, will not be obtained and the subject will not be eligible for the study.

All relevant information on the study will be summarized in an integrated subject information sheet and informed consent form (ICF). Based on this subject information sheet, the Investigator or designee will explain all relevant aspects of the study to each subject and guardian, if applicable, prior to entry into the study (i.e., any study-specific data is recorded on study-specific forms).

Each subject and guardian, if applicable, will have ample time and opportunity to ask questions and will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision. Only if the subject and/or guardian agrees to sign the ICF and has done so, may the subject enter the study. Additionally, the Investigator will personally sign and date the form. The subject or guardian, if applicable, will receive a copy of the signed and dated ICF. An assent section will be included as part of the ICF for obtaining assent from children age 7-17.

The ICF and any other written information provided to subjects and guardians will be revised whenever important new information becomes available that may be relevant to the subject's consent, or there is an amendment to the protocol that necessitates a change to the content of the subject information and/or the written ICF.

7.1.2 Questions regarding criteria

The subject will be asked questions to determine fulfillment of inclusion and exclusion criteria as specified in sections 5.1 and 5.2. Eligible subjects will be enrolled.

7.1.3 Baseline questions

Enrolled subjects will answer questions about demographics, including age, race, ethnicity, and sex, as well as questions about their relevant medical history.

7.2 Image collection

7.2.1 Skin locations

The skin to be imaged may be healthy or have suspected or known disease. The skin of the eyelid may not be imaged.

7.2.2 Preparation

The skin to be imaged will be cleaned using an alcohol swab to remove dirt and any other debris. The skin may be marked with a skin marker or targeting accessory. A small amount of pharmaceutical-grade mineral oil, alcohol-based hand sanitizer, ultrasound gel, or other pharmaceutical-grade immersion fluid may be applied to the skin. Subjects may be asked to remove any electronic wearables (e.g: smart watches, fitness tracker, hearing aid) etc. before we begin imaging.

7.2.3 Image and video collection

We may capture macroscopic images of the subject using a commercially available camera to capture the location and context of the skin to be imaged with the device. We may also image skin using a commercially available dermatoscope to accurately capture skin tone and other macroscopic skin features. Dermatoscope imaging may occur either before or after applying immersion fluid depending on the type of dermatoscope used. Imaging with the *Vio*^{TM} will begin after skin preparation. Each *Vio*^{TM} image and video requires only a few seconds to acquire. Macroscopic imaging, dermatoscope imaging, and *Vio*^{TM} imaging may occur in any order. Participants may volunteer for more than one imaging location (*e.g.*, the cheek, the forearm, the hand) in a single session. Steps 7.2.2 and 7.2.3 may be repeated for different imaging locations.

7.2.4 Subject feedback

Subjects may be asked to provide verbal feedback about the procedures, the device, their experience with standard dermatology care, and/or their opinions of hypothetical situations of how the device could be used in the future.

7.3 Completion

The subject may choose to end his/her participation at any time by notifying the Investigator(s) or designee, who will immediately stop image collection. In order to minimize bias in the collection of study data, information already collected may be included in data analysis.

The successful completion of the procedures, or subject withdrawal or termination of procedures, will be documented at the end of the subject's participation. Subject participation may occur over multiple visits, as subjects are allowed to participate more than once.

Subjects may be compensated for their participation up to $50 per session.

7.4 Photographs of subjects (optional procedure)

Images or partial images of subjects may be collected during the procedures listed here. These may be used to demonstrate the clinical ease of use of the technology. These images may be used in publications and scientific meetings. Any identifying features, e.g., faces of subjects, will be obscured in any published images.

[redacted]

7.6 Device Accountability and Traceability

[redacted]

All investigational devices will be stored at the investigational site in accordance with Good Clinical Practice (GCP) requirements and will be inaccessible to unauthorized personnel. Special storage conditions, if applicable, and a complete record of lot numbers and expiry dates can be found in the study file. Receipt, distribution, and destruction (if any) of the study device must be properly documented. Investigator(s) will be required to keep device accountability logs to ensure that devices are only used in properly consented subjects.

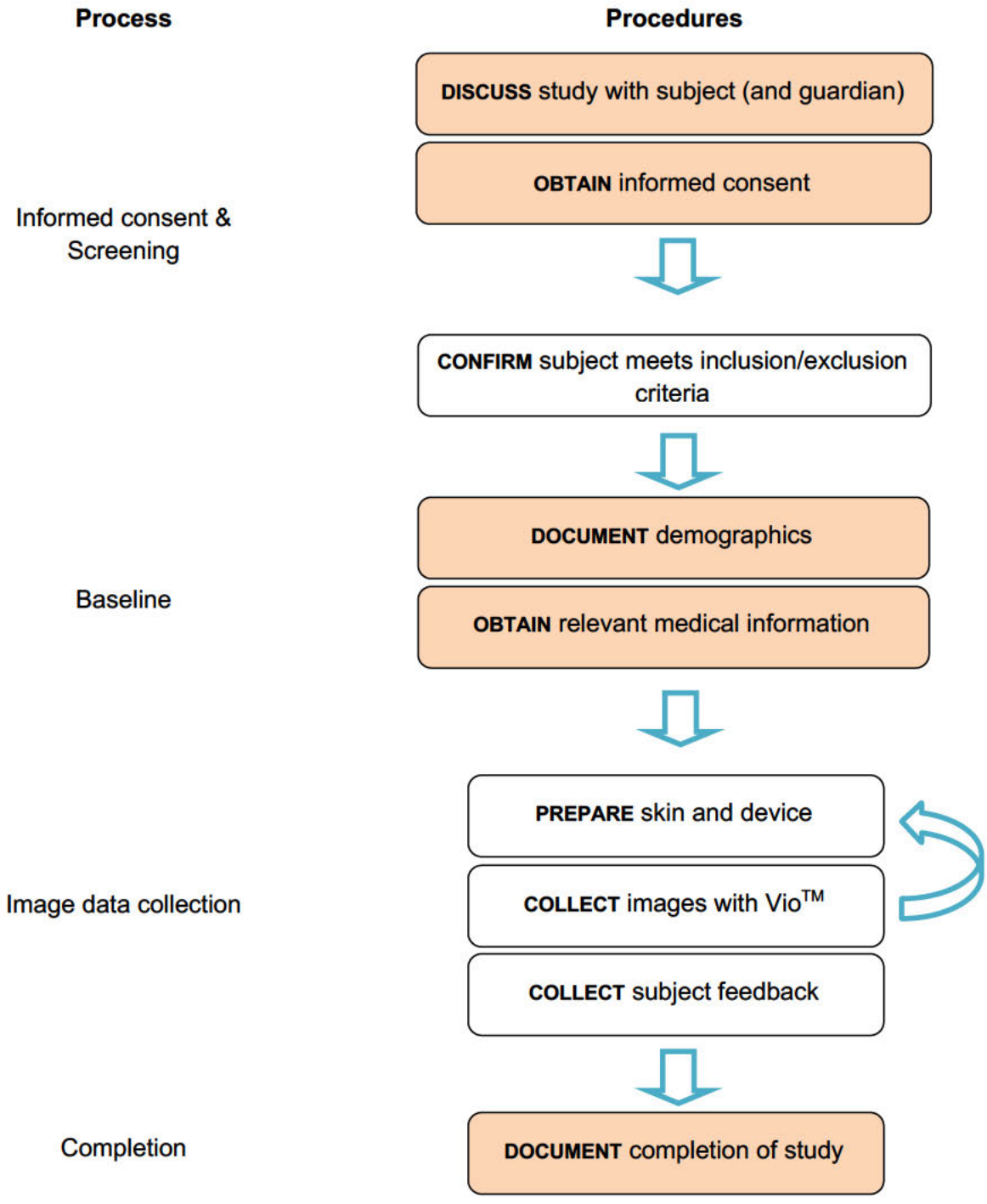


Figure 3. Schematic of procedures. The procedures outlined here are expected to typically take 1 hour, but no more than 3 hours.

8 Statistical procedures

8.1 Sample size determination

Although nonlinear imaging of human skin has been performed for years, little statistically meaningful data has been gathered to justify a robust sample size determination. A sample size of 120 was chosen to provide options for imaging skin of different demographics and minor technology modifications, although there are no strict requirements on enrollment of different groups for this protocol [2 (technology versions) × 2 (sex; male, female) × 5 (age; younger children, older children, young adults, middle aged adults, older adults) × 5 (race/ethnicity; latino/a, black, Indian, Asian, white) = 100+(overhead)=120] . The sample size calculation for this study is justified because this study is purely observational.

8.2 Statistical methods

The primary analysis involves the quantification of imaging performance of the *Vio*^{TM} as a function of skin type, location (*e.g.*, forearm, ear, nose), and health of skin. Because group sizes are small, statistical comparisons are unlikely to be significant. Still, data from this study will be important for planning statistically robust future studies. As a secondary aim, image performance will also be qualitatively evaluated by physicians trained in dermatopathology interpretation. Interim analyses are not planned.

8.3 Subject population(s) for analysis

All eligible subjects that are imaged will be included in the primary analysis according to the Aims outlined in Section 3. Non-primary analyses such as subject response to the procedure, occurrence of image artifacts, or typical reasons for screen failures may be reported for all subjects who consented to the study, or any defined subset of consented subjects.

9 Safety and adverse events

9.1 Definitions

Adverse event

An adverse event (AE) is any untoward medical occurrence (i.e., any unfavorable and unintended sign [including abnormal laboratory findings], symptom, or disease) in a clinical investigation subject after providing written informed consent for participation in the study. Therefore, an AE may or may not be temporally or causally associated with the use of the device.

A surgical procedure that was planned prior to the start of the study by any physician treating the subject should not be recorded as an AE.

In the following differentiation between medical history and AEs, the term “condition” may include abnormal physical examination findings, symptoms, diseases, laboratory findings, and electrocardiogram findings.

- Conditions that started before signing of informed consent and for which no symptoms or treatment are present until signing of informed consent are recorded as medical history (e.g., seasonal allergy without acute complaints).

- Conditions that started before signing of informed consent and for which symptoms or treatment are present after signing of informed consent, at *unchanged intensity*, are recorded as medical history (e.g., allergic pollinosis).
- Conditions that started or deteriorated after signing of the ICF will be documented as AEs.

Adverse device effect
An adverse device effect (ADE) is an AE related to the use of the device, including AEs resulting from insufficient or inadequate instructions for use, installation or operation, or any malfunction of the investigational medical device, use error or from intentional misuse of the medical device. The term is synonymous with device-related adverse event.

Device deficiency
A device deficiency is an inadequacy of device related to its identity, quality, durability, reliability, safety, or performance, such as malfunction, misuse, or use error and inadequate labeling.

Device failure
A device failure is a failure of the device to perform or function as intended, including any deviations from the performance specifications, or intended use.

Device malfunction
A device malfunction is a failure of the device to meet its performance specifications or otherwise perform as intended when used in accordance with the IFU (User Manual).

Device misuse
A device misuse occurs when the Investigator or designee uses the device in a manner that is contradictory to the IFU (User Manual). A device misuse will not be considered a malfunction.

Device-related adverse event
A device-related adverse event is any AE for which a causal relationship between the device and the AE is at least a reasonable possibility (i.e., the relationship cannot be excluded). The term is synonymous with adverse device effect (ADE).

Near incident
A near incident is any malfunction or deterioration in the characteristics and/or performance of the device (an ADE) which might have led to death or serious deterioration in health. The incident occurred and is such that if it occurred again, it might lead to death or serious deterioration in health.

Serious adverse device effect
An ADE that results in any of the consequences of a SAE. The term serious adverse device effect (SADE) is synonymous with “incident”.

Serious adverse event (SAE)
Any untoward medical occurrence that, at any dose, meets any of the following criteria (a—g):

a. Results in death.

b. Is life-threatening.

   The term 'life-threatening' in the definition refers to an event in which the subject was at risk of death at the time of the event, it does not refer to an event which hypothetically might have caused death if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization.

   A hospitalization or prolongation of hospitalization will not be regarded as an SAE if at least one of the following exceptions is met:

   - The admission results in a hospital stay of less than 12 hours.
   - The admission is pre-planned
     (i.e., elective or scheduled surgery arranged prior to the start of the study).
   - The admission is not associated with an AE
     (e.g., social hospitalization for purposes of respite care).

   However, it should be noted that invasive treatment during any hospitalization may fulfill the criterion of 'medically important' and as such may be reportable as an SAE dependent on clinical judgment. In addition, where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedence.

d. Results in persistent or significant disability / incapacity.

   Disability means a substantial disruption of a person's ability to conduct normal life's functions.

e. Is a congenital anomaly / birth defect.

f. Results in fetal distress, fetal death or a congenital abnormality or birth defect.

g. Is another medically important serious event as judged by the Investigator.

Unanticipated adverse device effect

An unanticipated adverse device effect (UADE) is any SADE 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 or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

9.2 Recording and reporting of adverse events

9.2.1 Classifications for adverse event assessment

All AEs will be assessed and documented by the Sponsor-Investigator according to the categories detailed below:

a) Seriousness: For each AE, the seriousness must be determined according to the criteria given in Section 9.1 (*Definitions*).

b) Intensity: The intensity of an AE is classified according to the following categories:

- Mild
- Moderate

- Severe

c) Causal relationship: The assessment of the causal relationship between an AE and the device or procedure is a clinical decision based on all available information at the time of the completion of the CRF. The assessment is based on the question whether there was a "reasonable causal relationship" to the procedure and/or device.

- Possible responses to causal relationship are "yes", "possibly", or "no".
- An assessment of "no" would include (1) the existence of a clear alternative explanation (e.g., the subject develops a local infection from an unrelated laceration) or (2) non-plausibility (e.g., the subject is struck by an automobile when there is no indication that the device caused disorientation that may have caused the event).
- An assessment of "possibly" indicates that there is a reasonable suspicion that the AE is associated with the procedure and/or device, but not definitively so.
- An assessment of "yes" indicates that the AE is very likely associated with the procedure and/or device.
- Important factors to be considered in assessing the relationship of the AE to the device or procedure include (1) the temporal sequence from the procedure—the event should occur after the procedure is initiated, (2) Underlying, concomitant, intercurrent diseases—each event should be evaluated in the context of the natural history and course of the procedure and any other medical conditions the subject may have, and (3) concomitant medications or procedures—any drugs the subject is taking or the additional procedures the subject receives should be examined to determine whether any of them may be suspected to cause the event in question.
- If the AE is assessed as related, it must be determined if the AE was associated with the device itself or with the procedure.

Causal relationship to other protocol-required procedure(s): The assessment of a possible causal relationship between the AE and other protocol-required procedure(s) is based on the question of whether there was a "reasonable causal relationship" to any protocol-required procedure(s). This category does not apply to AEs that are assessed by the Sponsor-Investigator as being related to the device itself or to the actual use of the device. Possible answers are "yes", "possibly", or "no."

d) Outcome: The outcome of the AE is to be documented as follows:

- Recovered/resolved
- Recovering/resolving
- Recovered/resolved with sequelae
- Not recovered/not resolved
- Fatal
- Unknown

9.2.2 Assessments and documentation of adverse events

The Sponsor-Investigator must collect all AEs for each subject from the time of the start of device procedure preparation up to 1 week following the end of subject study participation, whether or

not deemed related to the investigational device or procedure. All AE data will be recorded on the CRF.

All AEs will be followed until the event has subsided or, in case of permanent impairment, until the event stabilizes, and the overall clinical outcome has been ascertained.

The Sponsor-Investigator must document all device failures, malfunctions, and use errors, including the assessment whether the event is considered a near incident (ADE) using the CRF.

9.2.3 Reporting of serious adverse events and near incidents

The definition of SAE is provided in Section 9.1. These include device deficiencies that may have led to a SAE if a) suitable action had not been taken or b) intervention had not been made or c) if circumstances had been less fortunate. These are handled under the SAE reporting system.

- Notification of the IRB: Notification of the IRB about all relevant events (e.g., SAEs, near incidents, UADEs, device failures, device malfunctions) will be performed by the Sponsor-Investigator according to all applicable requirements.
- Notification of the authorities: The processing and reporting of all relevant events (e.g., SAEs, near incidents, UADEs, device failures, device malfunctions) to the authorities will be done by the Sponsor-Investigator according to all applicable regulations.

9.2.4 Expected adverse events

For this study, the applicable reference document is the most current version of the Risk analysis for the *Vio*^{TM} documented in Enspectra Health's quality system. The expectedness of AEs will be determined by the Sponsor-Investigator according to the applicable reference document and according to all local regulations. See the Section 1.4.2 for a list of expected adverse events.

9.3 Randomization codes and unblinding procedures

All enrolled subjects will undergo imaging with the *Vio*^{TM}. Subject group, therefore, will not be randomized nor blinded.

9.4 Stopping rules

Safety findings may trigger a temporary suspension of enrollment until an *ad hoc* safety review is conducted to determine whether the study should continue per protocol, proceed with caution, be further investigated, be discontinued, or be modified then proceed.

A safety review may be triggered by the occurrence of a UADE, or the occurrence or increased frequency of any situation the Sponsor-Investigator deems to be a trigger. Any findings shall be reported to the designated employees of Enspectra Health, who will then conduct a safety review and determine what steps will be taken.

The study may be discontinued at any time by the IRB, the FDA, or other government agencies as part of their duties to ensure that research participants are protected.

Participation of individual subjects may be terminated for multiple reasons, including failure to follow the Investigator's or designee's instructions, revelation of inaccurate information provided

during the screening process, safety reasons, subject is experiencing clear stress or pain (but the subject does not want to withdraw), or any scenario in which the Investigator judges it to be in the subject's best interest to terminate that subject's participation.

9.5 Medical monitoring

A medical monitor will be appointed for the study. The medical monitor will be a physician who is not involved in conducting the study and who has no or minimal conflict of interest. The medical monitor is responsible for ongoing monitoring of adverse event reports to identify safety concerns quickly. The medical monitor may also serve as a resource to the Sponsor-Investigator for advice about management of adverse events.

10 Data handling and record keeping

10.1 Confidentiality

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

Subject names will not be supplied to any people or organizations outside of Enspectra Health or authorized study staff. Within Enspectra Health, only the designated custodian(s) of the data shall have access to the subject names. Only the subject code will be recorded in the CRF. Study findings stored on a computer will be stored in accordance with local data protection laws. As part of the informed consent process, the subjects will be informed in writing that representatives of the Sponsor, IRB, or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

If the results of the study are published, the subjects' identities will remain confidential. The Sponsor-Investigator will maintain a list to enable subjects to be identified.

10.2 Source documents

Screening information, baseline information, recording of data, and information about subject completion of the study will be recorded directly on the CRF. As needed, information about device deficiency or malfunction, protocol deviations, or adverse events will also be recorded on the CRF. The CRFs will serve as the source documents for this study.

Image and video files will be gathered and stored in electronic format.

10.3 Records retention

Essential documents shall be archived by the Sponsor-Investigator safely and securely in such a way that they are readily available upon authorities' request for at least 2 years following completion of the study.

11 Study monitoring, auditing, and inspecting

The Sponsor-Investigator will verify that subject rights and well-being are protected. The Sponsor-Investigator (Enspectra Health) designates a hired clinical consultant to perform clinical monitoring,

including review of the CRF, before initiation and after completion of the study. The Sponsor-Investigator will allow direct access to all relevant subject files for the purpose of verifying entries made in the CRF and assist with the monitor's activities as required.

In accordance with applicable regulations, GCP, and Sponsor-Investigator procedures, the monitor will, prior to the start of the study, review with the Sponsor-Investigator representative the protocol, study requirements, and the Sponsor-Investigator's responsibility to satisfy regulatory and ethical requirements. When reviewing data collection procedures, the discussion will also include identification and documentation of source data items.

The monitor will verify that:

- The data are authentic, accurate, and complete.
- The safety and rights of the subject are being protected.
- The study is conducted in accordance with the currently approved protocol (including study treatment being used in accordance with the protocol.)
- Any other study agreements, GCP, and all applicable regulatory requirements are met.

Audits and inspections by regulatory health authority representatives and IRB are possible. The Sponsor-Investigator agrees to allow the auditor or inspector direct access to all relevant documents and allocate time to the auditor/inspector to discuss findings and any issues. Audits and inspections may occur at any time during or after completion of the study.

12 Premature termination of the study

The Sponsor has the right to close this study at any time, which may be due but not limited to the following reasons:

- If risk-benefit ratio becomes unacceptable owing to, for example
    - Safety findings from this study (e.g., SAEs).
    - Results of any interim analysis.
    - Results of parallel clinical studies.
    - Results of parallel animal studies
- If the study conduct (e.g., recruitment rate; drop-out rate; data quality; protocol compliance) does not suggest a proper completion of the trial within a reasonable time frame.
- Sponsor decision to suspend or discontinue evaluation of *Vio*^{TM}.
- Failure of the Investigator to comply with the tenets of the Study protocol, Study Investigator Agreements and Contracts, Regulatory requirements, or any applicable laws.
- Intentional submission of false study-related data and/or information by the research facility to the Sponsor.
- Sufficient data collected with smaller sample size

The Investigator will discuss with subjects any standard of care for future medical follow-up that may be required.

The Investigator has the right to close his/her center at any time.

For any of the above closures, the following applies:

- Closures should occur only after consultation between involved parties. Final decision on the closure must be in writing.
- All affected institutions (e.g., IRB(s), competent authority(ies), study center, head of study center) must be informed as applicable according to local law.
- All study materials (except documentation that has to remain stored at site) must be returned to the Sponsor. The Investigator will retain all other documents until notification given by the Sponsor for destruction.
- In case of a partial study closure, ongoing subjects, including those in post study follow-up, must be taken care of in an ethical manner.

Details for individual subject's withdrawal can be found in Section 5.4.

13 Ethical considerations

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the Sponsor-Investigator abide by GCP guidelines and guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in keeping with applicable local law(s) and regulation(s), including ISO 14155.

Documented IRB approval will be obtained before the start of the study, according to GCP, local laws, regulations and organizations. When necessary, an extension, amendment, or renewal of the IRB approval must be obtained. The IRB must supply to the Sponsor-Investigator, upon request, a list of the IRB members involved in the vote and a statement to confirm that the IRB is organized and operates according to GCP and applicable laws and regulations.

Strict adherence to all specifications laid down in this protocol is required for all aspects of study conduct; the Sponsor-Investigator may not modify or alter the procedures described in this protocol without proper IRB approval.

The Sponsor-Investigator, however, may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to the trial subjects without prior IRB approval. As soon as possible, the implemented deviation or change, the reasons for it, and if appropriate the proposed protocol amendment should be submitted to the IRB. Any deviations from the protocol must be explained and documented by the Sponsor-Investigator.

14 Publication Policy

The Sponsor-Investigator will evaluate the results of the trial and determine whether publication is appropriate, based on the investigational nature of the device and the early stage of research.

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