Enspectra Health, Inc.

Study Protocol

Study Title: VIO Imaging for Skin Tissue Assessment (VISTA) – a prospective, multicenter investigation of the VIO device in subjects undergoing routine skin biopsy

NCT #: NCT05619471

Document Date (IRB Approval Date): 15-March-2023

CLINICAL INVESTIGATIONAL PLAN

VIO Imaging for Skin Tissue Assessment (VISTA) – a prospective, multicenter investigation of the VIO device in subjects undergoing routine biopsy

Sponsor:	Enspectra Health, Inc. 2495 Hospital Dr, Suite 300 Mountain View, CA 94040 [redacted]
Study Device:	VIO A noninvasive skin imaging device that uses reflectance confocal and multiphoton microscopy to visualize skin
Study Title:	VIO Imaging for Skin Tissue Assessment (VISTA) – a prospective, multicenter investigation of the VIO device in subjects undergoing routine skin biopsy
Protocol Number:	[redacted]

1 Investigator Study Acknowledgment

Read and initial below.

____ I understand this protocol contains information that is confidential and proprietary to Enspectra Health, Inc.

____ Any additional information added to this protocol is also confidential and proprietary to Enspectra Health, Inc. and must be treated in the same manner as the contents of this protocol.

____ I have read the entire protocol.

____ I understand what the protocol asks me to do as an Investigator.

____ I will conduct this study following this protocol and will make a reasonable effort to complete the study in the time noted.

____ I will conduct this study in compliance with the principles of the Declaration of Helsinki, current Good Clinical Practices (GCP) guidelines, the IDE regulations (21 CFR §812) and all applicable regulatory requirements.

____ I will provide this protocol to study staff under my direct supervision. My study staff will keep the protocol and associated documents confidential.

____ Voluntary informed consent will be given by every subject or legally authorized representative prior to the initiation of any study-related procedure.

____ I will discuss this information with the study staff to ensure they are fully informed about the study protocol and the test article.

____ I will not start enrolling in this study until it is approved by a governing Institutional Review Board and I have received the necessary training by the study Sponsor.

____ I understand the study may be terminated or enrollment suspended at any time by Enspectra Health, Inc., with or without cause, or by me if it becomes necessary to protect the rights, safety or welfare of the study subjects.

_____

Name of Investigator

_____ _____

Investigator Signature Date

[redacted]

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[redacted]

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2 List of Abbreviations

2PA: 2-photon autofluorescence
ADE: Adverse device effect
AE: Adverse event
BCC: Basal cell carcinoma
BR: Blinded reader
COC: Certificate of Confidentiality
CPT: Current Procedural Terminology
CR: Comparative reader
CRF: Case report form
DD: Device deficiency
FDA: Food and Drug Administration (United States)
GCP: Good Clinical Practices
GLMM: Generalized linear mixed model
H&E: Hematoxylin and eosin
ICF: Informed Consent Form
IFU: Instructions For Use (e.g., User Manual)
IRB: Institutional Review Board
LAR: Legally Authorized Representative
MA: Medical assistant
MPM: Multiphoton microscopy
PA: Physician's assistant
PD: Protocol deviation
PHI: Protected Health Information
QA: Quality assurance
RCM: Reflectance confocal microscopy
RN: Registered nurse
SADE: Serious adverse device event
SAE: Serious adverse event
SCC: Squamous cell carcinoma
SHG: Second harmonic generation
UADE: Unanticipated adverse device effect
US: United States
VISTA: VIO Imaging for Skin Tissue Assessment

3 Study Summary

Title	VIO Imaging for Skin Tissue Assessment (VISTA) – A prospective, multicenter investigation of the VIO device in subjects undergoing routine skin biopsy
Protocol Number	[redacted]
Study Design	A prospective, multicenter, single-arm clinical investigation with 3 stages: 1. Procurement of training samples and development of training materials 2. Procurement of test samples and validation of Enspectra Health Images with Ground Truth Pathology (comparative reader assessment) * 3. Performance testing (blinded reader assessment of Enspectra Health images) * *Training samples will not be used for validation or performance testing.
Study Duration	12 months
Study Center(s)	2 - 5 Study Sites
Objectives	1. To demonstrate the safety and effectiveness of the VIO device in obtaining *in vivo* images that show tissue features including epidermis, dermis, collagen, blood vessels, and/or pigment. 2. To demonstrate that the tissue features identified on the images obtained with the VIO device align with the corresponding pathology images procured from the skin biopsy. 3. To evaluate the ability of blinded readers to correctly identify tissue features on images obtained with the VIO device.
Primary Effectiveness and Safety Endpoint	1. 100% agreement and validation of specific tissue features on VIO images (epidermis, dermis, collagen, blood vessels and pigment) in comparison to gold standard pathology images in Comparative Reader assessment. 2. >90% agreement between Blinded Reader VIO image assessment and answer key developed from validation assessment. 3. Safety will be assessed as the incidence of all adverse events (analyzed by severity, seriousness, and relationship to the device and procedure) that occur through the 7-day follow-up period.
Secondary Endpoints	1) 90% inter-reader agreement 2) Evaluation of secondary histopathology characteristics
Study Population	Men and women ages 18 to 99 years, in the United States undergoing a routine skin biopsy.
Number of Subjects	A maximum of 65 subjects will be enrolled (approximately N=20 training, N=30 validation for a total of N=50). Additional N=15

	subjects may be enrolled in the study in order to meet comparable distribution of samples described in the protocol.
Inclusion and Exclusion Criteria	Inclusion Criteria: 1. The subject is between 18 and 99 years of age. 2. The subject or Legally Authorized Representative (LAR) is able and willing to provide written informed consent. 3. The subject is planning to undergo a routine skin biopsy. 4. The subject is willing and able to remain still for periods of up to 3 minutes to allow for image capture. 5. The subject or LAR has sufficient mental capacity to understand the informed consent form (ICF) and comply with the protocol requirements. Exclusion Criteria: 1. The subject has a general health condition or systemic disease that in the opinion of the physician, precludes them from trial participation. 2. The subject has a known allergy or increased skin sensitivity to silicone, adhesives, or glycerin. 3. The subject's lesion targeted for biopsy: a. Is located on the palms of the hands, soles of the feet, fingernails, or toenails. b. Has dense hair that will not be removed prior to the skin biopsy. c. Has clinically significant abraded or ulcerated skin with or without discharge. d. Is associated with a wound or skin condition that in the opinion of the physician precludes them from participation e. Is located in mucosal tissue (i.e., oral, nasal, etc.). f. Is on tattooed skin. g. Is on a skin formation too tortuous for the investigational device to access (e.g., skin tags.) h. Is located in the periorbital region or directly on the eyelid.
Investigational Device	VIO, the investigational device, is a noninvasive real-time skin imaging device that that can provide histology-like images of skin epithelial tissue with subcellular resolution and dye-free color contrast. The system uses a handheld reflectance confocal and multiphoton microscope that contacts skin [redacted], without damaging the tissue. [redacted]

Duration of subject participation	Single visit with a 7-day (± 3 days) follow-up phone call to assess for adverse events.

4 Introduction

This is a protocol for a human research study. The study described herein, *VIO Imaging for Skin Tissue Assessment (VISTA) - A prospective, multicenter investigation of the VIO device in subjects undergoing routine skin biopsy*, will be conducted in accordance with this protocol, Good Clinical Practice Regulations, (21 CFR Parts 50, 54, 56, and 812) and the study site's research policies and procedures. This study will be conducted at two (2) to five (5) study sites in the U.S and will enroll a maximum of 65 subjects. All procedures will be performed at the participating study sites under the IRB approved study protocol. All subjects will be screened and enrolled per the protocol inclusion and exclusion criteria and will undergo the study-defined follow-up visits at 7 (± 3) days. The study has three (3) specific objectives: 1) To demonstrate the safety and effectiveness of the VIO device in obtaining *in vivo* images that show tissue features including epidermis, dermis, collagen, blood vessels, and/or pigment. 2) To demonstrate that the tissue features identified on the images obtained with the VIO device align with the corresponding pathology images procured from the skin biopsy; and 3) To evaluate the ability of blinded readers to correctly identify tissue features on images obtained with the VIO device.

4.1 Background

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

4.3 Study Rationale

[redacted] Noninvasive visualization of epidermis, dermis, blood vessels, collagen, and pigment with the VIO device will provide adjunctive information to physicians that will facilitate their assessment of patients' skin. This study will allow for the collection of VIO images during routine office visits involving skin biopsy procedures enabling a 1:1 comparison and validation of images collected with the investigational device and ground truth pathology. Additionally, this study will enable the evaluation and interpretation of VIO images by dermatopathologists and/or dermatologists who are blinded to the pathology results.

[redacted]

6 Investigational Device: VIO

Enspectra Health has developed the VIO system, a noninvasive, real-time imaging device, that can provide histology-like images of skin epithelial tissue with subcellular resolution and dye-free color contrast. This approach uses a handheld reflectance confocal and multiphoton microscope that contacts skin [redacted] without damaging the tissue [redacted]

[redacted]

[redacted]

c. [redacted]

[redacted]

6.1 Principles of Operation

[redacted]

6.1.1 Skin and device preparation

Prior to imaging, the lesion of interest and uninvolved skin site to be imaged must be cleaned thoroughly with an alcohol swab or soap and water to ensure the skin is free of residual ink, lotion, makeup etc. as this may disrupt imaging. The center hole of the targeting sticker is then placed over the center of lesion or skin site and secured firmly in place. A drop of pharmaceutical grade immersion fluid such as glycerin is applied inside this targeting sticker. The tip of the imaging wand is cleaned with an alcohol swab and a single-use imaging cover is placed onto the imaging tip. The imaging cover is also cleaned with an alcohol swab prior to imaging.

6.1.2 Operating the VIO device

If imaging the face, patients may be instructed to close their eyes. The user contacts the tip of the device against the target skin area and uses the digital touchscreen interface to start scanning. Images will begin to appear in low-resolution at near video frame rate to assist with skin targeting. [redacted]

6.1.3 Imaging Time

[redacted]

[redacted] Study Investigator(s) or designee(s) may optionally collect more images for up to 30 total minutes of intermittent imaging.

6.1.4 Transport and placement

[redacted] The device can be positioned on a flat, stable, clean surface such as a countertop prior to use.

6.1.5 Cleaning

The single-use imaging cover and the single-use adhesive targeting stickers are the only components of the device that directly contact the subject's skin and will be discarded after use. The imaging hub, wand, and cable will be wiped down after usage on an individual subject using 70% isopropyl alcohol in accordance with CDC guidelines for disinfection of noncritical items in healthcare facilities.

6.1.6 Image selection for analysis

For each biopsy site the Investigator will review all of the image files associated with that biopsy site on the VIO device. From this group, a subset [redacted] of captured VIO images will be identified and marked for subsequent analysis.

6.1.7 Image storage and export

During image collection, VIO images are stored internally on the device. When the maximum capacity of internal storage is reached, the user will not be able to collect additional images on the device. A warning screen will appear, and the users will be prompted to transfer images to free up storage. Images can be exported to an external USB provided by the Sponsor.

All VIO images shall be stored locally at each trial site using an external hard drive or similar physical storage device such as USB. VIO de-identified images will be exported to a secure cloud storage site or service for data backup and Sponsor retrieval/access. Visualization of VIO files may be performed using the VIO device and/or on other devices with DICOM capabilities.

6.2 Device Users and Training

Device users may include dermatologists, dermatopathologists, MAs (Medical Assistants), PAs (Physician Assistants), RNs (Registered Nurses). All users will be trained and qualified on the following by Enspectra personnel:

1. Powering on/off the system
2. VIO software application for data capture (Clinical Interface)
3. Scanning and Capturing Images
4. Using the presentation remote
5. Image Quality and Troubleshooting
6. Review/Selection of Images
7. Cleaning Procedures for the device
8. Preparing subject's skin for imaging
9. Preparing the imaging wand for imaging

10. Study Specific Documentation

Training may be conducted on healthy volunteers and/or subjects recruited as a part of Sponsor's PILOT study. All training will be documented for each user and maintained in the training records.

7 Regulatory Status

The Sponsor has determined that the "VIO" investigational device is a nonsignificant risk device under 21 CFR §812.2(b) based on the rationale provided below.

1. The device is not an implant, nor does it present a potential for serious risk to the health, safety, or welfare of a subject.
    - *The VIO device is not an implant, and it does not present a potential for serious risk to the health, safety or welfare of a subject.* [redacted]
2. The device is not purported or represented to be used for supporting or sustaining human life.
    - *The Enspectra Health device will be utilized for evaluating the external skin as an adjunct to standard visual assessments performed by physicians. It is intended for use in conjunction with standard of care biopsy procedures and is not used to support or sustain human life. Device usage is optional / elective and is not intended for diagnostic use or as a replacement for skin biopsy procedures. The device provides additional information to the physician during routine visual skin examinations.*
3. The device is not intended for a use of substantial importance in diagnosing, curing, mitigating, or treating disease or otherwise preventing impairment of human health; and,
    - *The device is used to evaluate the external skin as an adjunct to standard visual assessments performed by physicians. It is intended for use in conjunction with routine biopsy procedures and is not intended for diagnostic use or as a replacement for routine skin biopsies. Device usage is optional / elective and is not of a substantial importance in diagnosing disease. The device provides additional information to the physician during routine visual skin examinations.*
4. The device does not otherwise present a potential for serious risk to the health, safety, or welfare of the subject.
    - [redacted] *No reports of any adverse events have been observed.*

Also, in a previously approved investigational protocol [redacted] evaluating early iterations of the VIO device in a similar clinical assessment, the Salus IRB confirmed that the device was a nonsignificant risk device. Collectively, this information supports an NSR determination for the VIO investigational device under this study protocol.

8 Study Objectives and Endpoints

8.1 Study Objectives

- To demonstrate the safety and effectiveness of the VIO device in obtaining *in vivo* images that show tissue features including epidermis, dermis, collagen, blood vessels, and/or pigment.
- To demonstrate that the tissue features identified on the images obtained with the VIO device align with the corresponding pathology images procured from the skin biopsy.
- To evaluate the ability of blinded readers to correctly identify tissue features on images obtained with the VIO device.

8.2 Primary Endpoints (Safety and Effectiveness)

- 100% agreement and validation of specific tissue features on VIO images (epidermis, dermis, collagen, blood vessels and pigment) in comparison to gold standard pathology images in Comparative Reader assessment.
- >90% agreement between Blinded Reader VIO image assessment and answer key developed from validation assessment.
- Safety will be assessed as the incidence of all adverse events (analyzed by severity, seriousness, and relationship to the device and procedure) that occur through the 7-day follow-up period.

8.3 Secondary Endpoints

- 90% inter-reader agreement
- Evaluation of secondary histopathology characteristics

9 Study Design

This study is a prospective, multicenter, single-arm clinical investigation with 3 stages:

1. Procurement of training samples and development of training materials
2. Procurement of test samples and validation of Enspectra Health Images with Ground Truth Pathology (comparative reader assessment) *
3. Performance testing (blinded reader assessment of Enspectra Health images) *

*Training samples will not be used for validation or performance testing.

10 Subject Selection

10.1 Study Population

The target study population includes men and women 18 – 99 years old in the United States undergoing a routine skin biopsy. A maximum of 65 subjects may be enrolled at 2-5 sites (training set: N=20 subjects; validation set: N=30 subjects; Total N=50). Additional 15 subjects may be enrolled in the study in order to meet comparable distribution of samples as described in the tables below. Samples will be collected across a representative range of skin conditions, Fitzpatrick Skin Types, anatomical locations, ages and genders that are expected in actual

clinical usage (see Tables 1 - 5). Eligible subjects include those undergoing routine skin biopsies that meet the inclusion/exclusion criteria.

10.2 Inclusion Criteria

- The subject is between 18 and 99 years of age.
- The subject or Legally Authorized Representative (LAR) is able and willing to provide written informed consent.
- The subject is planning to undergo a routine skin biopsy.
- The subject is willing and able to remain still for periods of up to 3 minutes to allow for image capture.
- The subject or LAR has sufficient mental capacity to understand the informed consent form (ICF) and comply with the protocol requirements.

10.3 Exclusion Criteria

- The subject has a general health condition or systemic disease that in the opinion of the physician, precludes them from trial participation.
- The subject has a known allergy or increased skin sensitivity to silicone, adhesives, or glycerin.
- The subject's lesion targeted for biopsy:

    a. Is located on the palms of the hands, soles of the feet, fingernails, or toenails.
    b. Has dense hair that will not be removed prior to the skin biopsy.
    c. Has clinically significant abraded or ulcerated skin with or without discharge.
    d. Is associated with a wound or skin condition that in the opinion of the physician precludes them from participation
    e. Is located in mucosal tissue (i.e., oral, nasal, etc.).
    f. Is on tattooed skin.
    g. Is on a skin formation too tortuous for the investigational device to access (e.g., skin tags.)

11 Study Phases

11.1 Overview

This investigational study includes two stages: 1) Image Acquisition (see section 11.2); and, 2) Image Analysis (see section 11.3). The Image Analysis Stage is comprised of the following 3 phases:
1) Training / Consensus
2) Validation (VIO image assessment in comparison with ground truth pathology) and establishment of an "answer key"
3) Performance Testing (VIO image evaluation by a group of readers blinded to pathology results). These phases are briefly described separately below.

11.2 Image Acquisition Stage

a. Two to five sites will enroll subjects undergoing routine biopsies that meet the inclusion / exclusion criteria. All sites will utilize their standard-of-care labs to obtain pathology slides for comparison with the VIO images.
b. Sites will enroll up to a maximum of N=65 subjects to complete enrollment for training (N=20 unique subjects) and validation sets (N=30 unique subjects).
c. The training set and validation set will have a comparable distribution of samples across subgroups of interest, and each set will contain samples representing a cross section of expected real-world variation as described below and in Tables 1 – 5 (Appendix A includes supportive statistics for these subgroup sample sizes):
    i. Skin conditions (BCC, SCC, benign/atypical conditions).
    ii. Skin types (Fitzpatrick Type I, II, III, IV-VI).
    iii. Anatomical location
    iv. Age
    v. Sex

Table 1. Conditions.[4]

Conditions	Composition in typical practice (%)	Expected Sample Number Range* *Training Set* *n = 20*	Expected Sample Number Range* *Validation Set* *n = 30*
Basal cell carcinoma	21.2	2 - 7	4 - 9
Squamous cell carcinoma (SCC), including in situ	16.7	1 - 6	2 - 8
Benign or atypical conditions	62.2	10 - 15	15 - 22

Table 2. Fitzpatrick skin type.[22]

Skin type	Composition in typical practice (%)	Expected Sample Number Range* *Training Set* *n = 20*	Expected Sample Number Range* *Validation Set* *n = 30*
Type I	12.4	1 - 4	1 - 6
Type II	34.9	4 - 10	7 - 14
Type III	37.3	5 - 10	8 - 15
Type IV-VI	15.4	1 - 5	2 - 7

Table 3. Lesion anatomical location.[23]

Skin type	Composition in typical practice (%)	Expected Sample Number Range* *Training Set* *n = 20*	Expected Sample Number Range* *Validation Set* *n = 30*
Head and neck	83	14 - 19	22 - 27

Limb	7	0 - 3	0 - 4
Torso	10	0 - 4	1 - 5

Table 4. Age.[24]

Age	Composition in typical practice (%)	Expected Sample Number Range* *Training Set* *n = 20*	Expected Sample Number Range* *Validation Set* *n = 30*
20 - 49	6.5	0 – 3	0 – 4
50 - 64	22.9	2 – 7	4 – 10
65+	69.8	11 – 17	18 – 24

Table 5. Sex.[24]

Sex	Composition in typical practice (%)	Expected Sample Number Range* *Training Set* *n = 20*	Expected Sample Number Range* *Validation Set* *n = 30*
Female	50	7 - 13	11 - 19
Male	50	7 - 13	11 - 19

*Sample ranges based on 10th (lower) and 90th (upper) percentiles from simulations (see Appendix A). Minimum value for the number of samples required in the study is the lower bound of the range (derived from 10th percentile). There is no maximum value for any sample type.

11.3 Image Analysis Stage

The Image Analysis stage includes the following three phases:

1. Development of consensus training materials by three (3) Comparative Readers
2. Validation of Enspectra Health Images with Ground Truth Pathology by three (3) Comparative Readers
3. Performance testing (Blinded Reader assessment of Enspectra Health images)

[redacted]

[redacted]

11.3.1 ***Phase 1: Training/Consensus***

1. Three Comparative Readers (CRs) will train as a group using the [redacted] VIO images and all available pathology slides. Comparative readers will assess a minimum of [redacted]. VIO images from 20 lesions on 20 subjects meeting the requirements described above in Tables 1-5 will be evaluated for this training assessment.
2. CRs will label the images [redacted]
3. Images will be marked as elements are identified but exhaustive annotation is not required. Samples will be reviewed until complete consensus achieved. Secondary histopathology characteristics will also be evaluated and recorded.
4. The output of this phase of the study will be a set of 20 training samples consisting of matched VIO images and ground truth pathology slides.

11.3.2 ***Phase 2: Validation*** (VIO Image Assessment in Comparison with Ground Truth Pathology) and Establishment of an "Answer Key"

1. A total of 30 VIO images from 30 subjects meeting the requirements described above in Tables 1-5 will be evaluated in this validation assessment.
2. Two experienced physicians with dermatopathology expertise will serve as the primary CRs. A third expert CR, with RCM experience, will adjudicate discrepancies between the two primary CRs.
3. Two primary CRs will assess the [redacted] VIO images and predict histology. [redacted]
4. The expert CR will review all pairs and will work with the two primary CRs until complete consensus achieved (i.e., until 100% VIO image v. ground truth pathology achieved across all 3 CRs). Achieving 100% agreement/consensus at the completion of this process represents success for Primary Endpoint 1: 100% agreement and validation of specific tissue features on VIO images (epidermis, dermis, collagen, blood vessels and pigment) in comparison to gold standard pathology images in Comparative Reader assessment.

5. The output of this validation process will establish the answer key for the performance testing assessment.

11.3.3 ***Phase 3: Performance Testing*** (VIO Image Assessment by Readers Blinded to Pathology Results)

1. Three Blinded Readers (BR) will be trained using the training set developed in Phase 1. Training materials may also be supplemented with other data sets [redacted]
2. The Phase 2 Validation Set and the answer key derived from such will be used for the BR assessment. BRs will assess [redacted] Assessment of performance of the BRs defines the success of Primary Endpoint 2: >90% agreement between Blinded Reader VIO image assessment and answer key developed from validation assessment.

12 Study Procedures

12.1 Study Schedule

Visit-1 (Procedure Day)	Visit-2 (Follow up)
Informed Consent	Assessment of AE
Screening (Eligibility Criteria)	Study Exit
Demographics and Medical History	
Imaging with VIO (Study Procedure)	
Assessments for AE, PD, DD (if any)	

12.2 Recruitment

Potential subjects will be recruited from the Investigator's practice, referrals, and local advertising. Any study-related advertisements will be approved by the governing IRB prior to use. Consecutive subjects meeting the inclusion and exclusion criteria will be recruited based on the guidelines described above in Tables 1 – 5.

12.3 Informed consent process

All subjects or LARs must be provided a consent form describing the study with sufficient information for subjects to make an informed decision regarding their participation. Subjects or LARs must sign the IRB approved informed consent prior to participation in any study specific procedure. The subject or LAR must receive a copy of the signed and dated consent document. The signed copy of the consent document must be retained in the subject's study file. A patient who is eligible and signs the study consent will be considered a study subject.

12.4 Screening

After the subject or LAR 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. The subject screening interview will collect demographic data and relevant medical history.

All subjects who are eligible with a signed informed consent form will be assigned a unique screening ID number. Only subjects who undergo device use will count towards the study's sample size.

12.5 Demographics and medical history

Demographic data to be collected will include date of birth, race, ethnicity, and Fitzpatrick skin type. Relevant medical history (i.e., previous diagnoses, diseases, or surgeries) will also be collected.

12.6 Procedure

a. Study staff will procure images of the planned biopsy site using a standard camera and dermatoscope
b. Trained device users will procure VIO *in vivo* images:
    i. at the planned biopsy site
    ii. at a contralateral (preferred) or nearby non-suspicious site of uninvolved-appearing tissue
c. Approximately 20-100 images will be taken for each planned biopsy site and 2-10 images will be taken for each uninvolved site. Approximately 4-8 representative images from the planned biopsy site will be selected for further review/assessment.
d. Physical H&E slides from the biopsy site may be converted into digital pathology images by the Investigator or the Sponsor using a commercially available scanner.
e. Subjects will be asked to answer a brief questionnaire about their opinions and/or experience

12.7 Follow-Up Phone Call

A phone call follow-up evaluation will be conducted 7 days (+/-3 days) after the study procedure to record adverse events.

12.8 Study Exit

After the 7-day (+/-3 days) follow-up, the subject's participation in the study is complete and the Investigator or designee(s) will complete the study exit CRF.

If an adverse event occurs the subject will be followed by the Investigator until the AE is resolved or for a minimum of 30 days after the subject's participation in the study is complete.

12.9 Early Withdrawal of Subjects

Subjects may be withdrawn from the study at any time 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
    - Inability to follow the Investigator's or designee's instructions
- Subject or LAR withdraws consent
- Device non-operational at the scheduled imaging time

13 Statistical Analysis Plan

13.1 Sample Size Determination

The sample sizes of 20 subjects for the training set and 30 subjects for the validation set were determined by simulation studies (see Appendix A). The goal of these studies was to select sample sizes with a high probability of representation in as many of the subgroups of interest as possible. These sample sizes provide a > 90% chance of representation for underlying conditions of interest and Fitzpatrick skin type groups. In addition, these sample sizes reliably provide variability across age groups, genders and lesion locations.

If areas are assumed to be independent, the sample size of at least 360 areas in the validation set will produce confidence intervals with a width of ± 6.2%. The areas are correlated, which decreases precision, but the number of areas is expected to be larger, which increases it. If these factors offset, a confidence interval width of ± 6.2% is a reasonable estimate of the expected results.

13.2 Statistical Analysis Methods

13.2.1 General Definitions

Subgroups of Interest

This study aims to recruit subjects and lesions across a range of demographic and clinical subgroups. The sample size was selected so that there was high probability of representation across the categories seen in clinical practice. Specifically, the subgroups of interest are:

- Underlying skin condition (BCC, SCC, other)
- Fitzpatrick skin type (I, II, III, IV-VI)
- Lesion location (head and neck, limb, torso)
- Age group (18 to 49, 50 to 64 and 65+)
- Sex (men, women)

[redacted]

[redacted]

[redacted]

[redacted]

13.2.2 Analysis of Training Set

[redacted]

Descriptive statistics only will be produced for the entire training set of VIO images and full histopathology slides. The purpose of this summary is to describe the training sample data set, and to verify that the training set is similar to the validation set. As such, the number of patients, average number of lesions within subject, and average number of photos within subject will be summarized using n, mean, standard deviation and range. In addition, the distribution of lesions across relevant subgroups will be tabulated (see section 11.2). The distribution of primary histopathology characteristics observed in the training set will also be tabulated. The incidence of secondary histopathology characteristics observed in the training set will also be summarized.

[redacted]. Since this phase will continue until complete consensus is achieved for these images, no analysis of this process will be performed. [redacted]

[redacted]

[redacted]

[redacted]Other materials may be used to train Blinded Readers at the CRs' discretion, including but not limited to third party publications, data from VIO prototype devices, and VIO images from uninvolved skin sites. No Validation Set data may be used for training.

13.2.3 Comparison of Validation Set to Ground Truth Histology

[redacted]

First, an expert reviewer and 2 comparative reviewers will assess each of the VIO images, blinded to the histopathology results. For each area, the reviewer will classify the predicted primary histopathology characteristics according to the categories described in section 11.4. Second, the expert reviewer will determine the actual primary histopathology characteristics based on the histopathology results. Third, the predicted characteristics will be compared to the expert reviewer classification.

An iterative review process will be performed until there is complete consensus across all reviewers. The output of this iterative review process will be an "answer key" that reflects 100% consensus of the comparative results from the VIO image and pathology across all 3 comparative readers. Achieving 100% agreement/concensus through this process satisfies the first Primary Endpoint.

13.2.4 Performance Testing of Blinded Readers

Once the answer key is finalized for the validation set, 3 blinded readers will be used for performance testing. The blinded readers will be trained using the training set, and will then be provided with the VIO images only from the validation set. Each blinded reader will classify the primary histopathology features and regions for each of the approximately 360 areas in the validation set and identify secondary histopathology characteristics in each VIO image. Accuracy will be assessed for primary histopathology characteristics only, compared to the answer key, as the proportion of total points for each of the blinded reviewers as described in section 11.5.

The accuracy assessment will be performed using a conditional, generalized linear mixed model (GLMM) to account for correlation among multiple images obtained for the same subject, and for correlation of responses within each reviewer. Once each response has been scored, the data will be condensed to 1 record per patient per reviewer. The dependent variable will be the number of points obtained divided by the maximum number of points possible. The GLMM will use a binomial response distribution and a logit link function. A compound symmetry variance-covariance matrix will be used to account for correlation. Random effects will be included for each subject-reviewer cluster. Reviewer will be included as a model covariate, and the correlation-adjusted proportion correct will be calculated separately for each reviewer by applying the standard logistic function to the least-square means by reviewer. This GLMM framework (with an intercept term) will be used to produce overall and reviewer-specific point estimates and 95% confidence intervals. The point-estimate target for overall accuracy is 90%.

Inter-rater agreement among the BRs will be assessed using Kappa statistics, and the target for agreement is 90%. Overall accuracy compared to the answer key across the subgroups will be assessed by adding each subgroup to the GLMM as a categorical variable. Accuracy for secondary histopathology characteristics may also be reported, but there are no *a priori* targets.

13.3 Subject Population(s) for Analysis

Analysis populations will differ based on the purpose of the analysis. The Safety Analysis Set will include all subjects for whom an attempt was made to image lesions using the VIO device. Adverse events, protocol deviations (PDs), device malfunctions and study disposition will be reported for this set. The Full Imaging data set will include all subjects in the safety set and all VIO images. The Primary Analysis Set will include only subjects with at least 4 images of sufficient quality to be included in the training or validation set, and only images of sufficient quality to be included in these sets. The Primary Analysis Set will be used for all other descriptive summaries and for analyses of accuracy and agreement. A comparison between the Full Imaging Set and the Primary Analysis Set will be used to summarize the rate of high-quality images.

14 Safety and Adverse Events

14.1 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 written informed consent has been provided for participation in the study. Therefore, an AE may or may not be temporally or causally associated with the use of the device.

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.

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' 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 (UADE)

An unanticipated adverse device effect (UADE) is any SAE, 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.

AE Severity and Relatedness

Each AE occurring in the study will be characterized by the study Investigator as to severity and relatedness.

AE Severity Grading System.

Severity Grade	**AE Description**
Mild	AE is transient and easily tolerated by the subject, even if it causes discomfort
Moderate	AE causes the discomfort and interrupts usual activities
Severe	AE causes considerable interference with usual activities and may be incapacitating or life-threatening

AE Relatedness Grading System. *

Grade	**Relationship of AE to study device**	**Description**
5	Definite	An event that follows a reasonable temporal sequence from administration of the study device; that follows a known or expected response pattern to the study device; and that is confirmed by improvement on stopping.
4	Probable	An event that follows a reasonable temporal sequence from administration of the study device; that follows a known or expected response pattern to the study device; and that is unlikely to have been caused by concurrent/underlying illness or other drugs, procedures, or other causes.
3	Possible	An event that follows a reasonable temporal sequence from administration of the study device; that follows a known or expected response pattern to the study device; but may have been caused by concurrent/underlying illness, drugs, procedure, or other causes.
2	Unlikely	An event that does not follow a reasonable temporal sequence from administration of the study device; that does not follow a known or expected response pattern to the study device, or most likely was caused by concurrent/underlying illness, drugs, procedure, or other causes, because of their known effects.
1	Not related	An event almost certainly caused by concurrent/underlying illness, drugs, procedure, or other causes.

*Note that an AE occurring before treatment with the study device will be categorized as unrelated to the study device.

14.2 Reporting of Adverse Events

14.2.1 Assessments and documentation of adverse events

The Investigator or designee must collect all AEs for each subject from the time of the start of device procedure preparation to 7 days (± 3 days) 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, for a minimum of 30-days after subjects' participation has ended.

14.2.2 Reporting of serious adverse events

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

14.3 Risks and Benefits

14.3.1 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 reflectance confocal and multiphoton microscopy to display clinically relevant features of cellular structure and skin architecture noninvasively.

14.3.2 Risks

The risks of this study and use of the device to image skin are considered to be no more than minimal. The VIO procedure described in this protocol is very similar to RCM imaging of skin with commercially available devices. [redacted]

- **Skin:** The VIO device takes an image of skin tissue by exciting and reflecting light off 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[26] so tissue is not damaged, while still providing the high instantaneous intensities needed for reflectance confocal and multiphoton microscopy. [redacted].[27]
- **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). As such, eye protection for the user is not be required.

The VIO procedure is also similar to commonly performed office-based dermoscopy procedures. Similar to dermoscopy, the VIO procedure involves non-invasive illumination of skin and magnification of skin structures. 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.[28] 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.[28]

14.4 Potential Risks to Study Subjects

The following are the possible risks associated with the VIO procedure:

- Irritation from adhesive targeting guide
- Allergic reaction such as irritation or sensitivity to immersion fluid or cover
- Pain due to pressure from the wand and/or sensitivity of the lesion
- Infection caused due to improper cleaning of the device

14.5 Risk Mitigation

VIO will limit the risk of allergic contact dermatitis by using an immersion fluid approved to be used in medical settings (e.g., pharmaceutical grade glycerin). Additionally, the VIO device comprises materials generally considered biocompatible when used in similar devices for similar applications (*e.g.*, silicone). The users will be trained thoroughly on device use to mitigate the risk of patient experiencing any pain during the sessions. [28]We will mitigate the risk of infection in this protocol by using a single use imaging cover and cleaning it with an alcohol swab prior to use.

14.6 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 conflict of interest. The medical monitor is responsible for ongoing monitoring of adverse event reports to identify safety concerns quickly.

15 Data Handling and Record Keeping

15.1 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 the Sponsor and Investigators. 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 and/or LARs 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. The data may also be used for future research, development and commercial activities and any identifying information will be kept confidential and described in the consent form. If the results of the study are published, the subjects' identities will remain confidential. The Investigator will maintain a list to enable subjects to be identified.

[redacted]

16 Device Malfunction

16.1 Definitions

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.

16.2 Recording and Addressing Device Malfunction

Throughout the study, the Investigator and study staff will report and document all device malfunctions related to the identity, quality, durability, reliability, safety, or performance of the device.

[redacted]

17 Study Monitoring, Auditing, and Inspecting

Representatives of the Sponsor will visit all study sites to perform monitoring and data management functions, and provide participating sites with relevant contact information, as necessary.

[redacted]

18 Ethical and Legal Considerations

18.1 Compliance with Good Clinical Research Practice

This study will be conducted in compliance with the principles of the Declaration of Helsinki, with the current Good Clinical Practice (GCP) guidelines and with other applicable regulations. The Investigator and all study staff will conduct the study in compliance with this protocol. Voluntary informed consent will be given by every subject or LAR prior to the initiation of any study-related procedures. The rights, safety and well-being of the study subjects are the most important considerations and prevail over the interests of science and society. All personnel involved in the conduct of this study must be qualified by education, training and experience to perform their assigned responsibilities.

18.2 Institutional Review Board (IRB) and Informed Consent

The IRB-approved consent form must include all elements required by FDA, state, and local regulations, and may include appropriate additional elements. Before study initiation, the Investigator must have written and dated approval from the IRB for the protocol, consent form, subject recruitment materials/process (e.g., advertisements), and any other written information to be provided to subjects or LARs. The Investigator should also provide the IRB with a copy of the product labeling, information to be provided to subjects or LARs and any updates. The Investigator will submit documentation of the IRB approval to the Sponsor. Copies of all correspondence with the IRB regarding this study must be sent to the Sponsor.

The Investigator or designee will explain the study to each potential subject and LAR and the subject or LAR must indicate voluntary consent by signing and dating the approved informed consent form. The Investigator or designee must provide the subject or LAR with a copy of the consent form in a language the subject or LAR understands. The Investigator will maintain documentation that informed consent was obtained prior to the initiation of any study-specific procedures. Any failure to obtain informed consent must be reported to the IRB and Sponsor.

Withdrawal of IRB approval must be reported to the Sponsor within 5 working days.

18.3 Protocol Compliance and Protocol Deviations

The Investigator and all designees will comply with the IRB-approved protocol. All deviations from the protocol must be documented. The Investigator or designee will notify the Sponsor immediately if a deviation from the protocol was required to protect patient safety.

[redacted]

[redacted]

18.11 Publication and Reporting of Study Results

The study will be registered on clinicaltrials.gov before the first subject is enrolled. [redacted]

[redacted]

[redacted]

[redacted]

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Appendix A: Sample Representation Across Patient and Lesion Characteristics

1. Purpose

To determine the chance of observing sufficient variability in subject and lesion characteristics that may impact reviewer ability to assess images. Specifically, the goal was to determine whether the planned sample sizes of 20 for the training set and 30 for the validation set were sufficient to observe representation of subgroups for underlying skin condition, Fitzpatrick skin type, lesion location, age group and sex with high probability.

2. Methods

Each characteristic was modeled separately. Frequency distribution estimates were obtained from recent literature. A simulation analysis was performed using SAS version 9.4. The simulation algorithm was as follows:

1. Simulate the appropriate number of subjects (20 for the training set or 30 for the validation set).
2. Assign subgroup categories using a multinomial distribution.
3. Count the number of subjects in each subgroup.

This process was repeated 10,000 times for each subject or lesion characteristic, and the count distribution over each subgroup level was examined. The minimum expected number was defined as the 10^{th} percentile, that is, out of 10,000 trials the chance of observing at least that many was 90%. The average count (empirical expected value) was also recorded.

3. Results

3.1. Underlying skin condition

There is a 90% chance (10th percentile) of seeing at least 1 SCC and 2 BCC lesions in the training set. There is a 75% chance (25th percentile) of seeing at least 2 SCC and 3 BCC lesions in the training set (Table 3.1.2).

There is a 90% chance (10th percentile) of seeing at least 2 SCC and 4 BCC lesions in the validation set. There is a 75% chance (25th percentile) of seeing at least 4 SCC and 5 BCC lesions in the validation set (Table 3.1.3).

Table 3.1.1. Underlying data by underlying skin condition.[4]

Group	**% of Skin Biopsy-Receiving Population**
Basal cell carcinoma (BCC)	21.2%
Squamous cell carcinoma (SCC)	16.7%
Other	62.2%

Table 3.1.2. Simulations for training set (N=20) by underlying skin condition.

Group	N sims	10th Pctl	EV	Std Dev	Min	1st Pctl	25th Pctl	Median	75th Pctl	90th Pctl	99th Pctl	Max
BCC	10,000	2	4.3	1.84	0	1	3	4	5	7	9	12
SCC	10,000	1	3.3	1.68	0	0	2	3	4	6	8	12
Other	10,000	10	12.4	2.17	4	7	11	12	14	15	17	20

Table 3.1.3. Simulations for validation set (N=30) by underlying skin condition.

Group	N sims	10th Pctl	EV	Std Dev	Min	1st Pctl	25th Pctl	Median	75th Pctl	90th Pctl	99th Pctl	Max
BCC	10,000	4	6.4	2.25	0	2	5	6	8	9	12	16
SCC	10,000	2	5.0	2.05	0	1	4	5	6	8	10	13
Other	10,000	15	18.6	2.69	9	12	17	19	20	22	25	27

3.2. Fitzpatrick Skin Type

There is a 90% chance (10th percentile) of seeing at least 1 Type I and 1 Type IV-VI lesion in the training set. There is a 75% chance (25th percentile) of seeing at least 1 Type I and 2 Type IV-VI lesions in the training set (Table 3.2.2).

There is a 90% chance (10th percentile) of seeing at least 1 Type I and 2 Type IV-VI lesions in the validation set. There is a 75% chance (25th percentile) of seeing at least 2 Type I and 3 Type IV-VI lesions in the validation set (Table 3.2.3).

Table 3.2.1. Underlying data by Fitzpatrick skin type.[22]

Group	% of Population Receiving Pigmented Skin Lesion Biopsy
Type I	12.4%
Type II	34.9%
Type III	37.3%
Type IV-VI	15.4%

Table 3.2.2. Simulations for training set (N=20) by Fitzpatrick skin type.

Group	N sims	10th Pctl	EV	Std Dev	Min	1st Pctl	25th Pctl	Median	75th Pctl	90th Pctl	99th Pctl	Max
Type I	10,000	1	2.5	1.47	0	0	1	2	3	4	6	9
Type II	10,000	4	7.0	2.14	0	2	6	7	8	10	12	16
Type III	10,000	5	7.4	2.15	1	3	6	7	9	10	12	15
Type IV-VI	10,000	1	3.1	1.60	0	0	2	3	4	5	7	9

Table 3.2.3. Simulations for Validation Set (N=30) by Fitzpatrick skin type.

Group	N sims	10th Pctl	EV	Std Dev	Min	1st Pctl	25th Pctl	Median	75th Pctl	90th Pctl	99th Pctl	Max
Type I	10,000	1	3.7	1.81	0	0	2	4	5	6	8	12
Type II	10,000	7	10.5	2.59	2	5	9	10	12	14	17	21
Type III	10,000	8	11.1	2.65	2	5	9	11	13	15	18	21
Type IV-VI	10,000	2	4.7	1.98	0	1	3	5	6	7	10	14

3.3. Lesion Anatomical Location

The chance of seeing at least 1 limb and torso lesion in the validation set is 75%. [note: it is unlikely that we end up with only head and neck lesions but there is a material chance we will missing at least 1 of the other locations] (Table 3.3.2).

There is a 90% chance of observing at least 1 torso lesion and a 75% chance of observing at least 1 limb lesion in the validation set. There is a 75% chance of observing at least 2 torso lesions in the validation set (Table 3.3.3).

Table 3.3.1. Underlying data by lesion anatomical location.[23]

Group	% of positive BCC diagnosis population
Head and neck (head)	83%
Hands, arms, feet, or legs (limb)	7%
Torso	10%

Table 3.3.2. Simulations for training set (N=20) by lesion anatomical location.

Group	N sims	10th Pctl	EV	Std Dev	Min	1st Pctl	25th Pctl	Median	75th Pctl	90th Pctl	99th Pctl	Max
Head	10,000	14	16.6	1.68	10	12	16	17	18	19	20	20
Limb	10,000	0	1.4	1.13	0	0	1	1	2	3	5	8
Torso	10,000	0	2.0	1.33	0	0	1	2	3	4	6	9

Table 3.3.3. Simulations for validation set (N=30) by lesion anatomical location.

Group	N sims	10th Pctl	EV	Std Dev	Min	1st Pctl	25th Pctl	Median	75th Pctl	90th Pctl	99th Pctl	Max
Head	10,000	22	24.9	2.05	16	20	24	25	26	27	29	30
Limb	10,000	0	2.1	1.40	0	0	1	2	3	4	6	9
Torso	10,000	1	3.0	1.62	0	0	2	3	4	5	7	10

[redacted]

3.4. Age

There is a 50% chance of observing a lesion in a 20- to 49-year-old in the training set, but a 90% chance of observing at least 2 lesions in patients age 50 to 64. There is a 75% chance of observing at least 3 lesions in patients age 50 to 64 in the training set (Table 3.4.2).
There is a 75% chance of observing a lesion in a 20- to 49-year-old in the validation set. There is a 90% chance of observing at least 4 lesions in patients age 50 to 64, and a 75% chance of observing at least 5 lesions in patients age 50 to 64 in the validation set (Table 3.4.3).

Table 3.4.1. Underlying data by age.[24]

Group	% of positive NMSC diagnosis population
20-49	6.5%
50-64	22.9%
65+	69.8%

Table 3.4.2. Simulations for training set (N=20) by age.

Group	N sims	10th Pctl	EV	Std Dev	Min	1st Pctl	25th Pctl	Median	75th Pctl	90th Pctl	99th Pctl	Max
20-49	10,000	0	1.3	1.11	0	0	0	1	2	3	4	7
50-64	10,000	2	4.6	1.87	0	1	3	4	6	7	9	12
65+	10,000	11	13.9	2.05	7	9	13	14	15	17	18	20

Table 3.4.3. Simulations for validation set (N=30) by age.

Group	N sims	10th Pctl	EV	Std Dev	Min	1st Pctl	25th Pctl	Median	75th Pctl	90th Pctl	99th Pctl	Max
20-49	10,000	0	1.9	1.34	0	0	1	2	3	4	6	10
50-64	10,000	4	6.9	2.33	0	2	5	7	8	10	13	16
65+	10,000	18	20.9	2.53	11	15	19	21	23	24	26	30

3.5. Sex

The chance that both sexes are represented in the training set is close to 100%. The chance of obtaining samples for at least 7 patients for each sex is 90% (Table 3.5.2).
The chance that both sexes are represented in the validation set is close to 100%. The chance of obtaining samples for at least 11 patients for each sex is 90% (3.5.3).

Table 3.5.1. Underlying data by sex.[24]

Group	% of positive NMSC diagnosis population
Female	50%
Male	50%

Table 3.5.2. Simulations for training set (N=20) by sex.

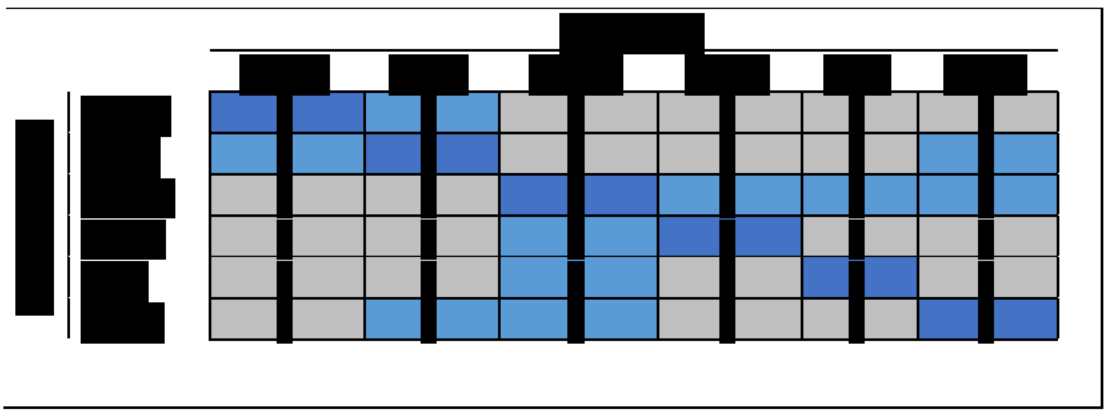
Group	N sims	10th Pctl	EV	Std Dev	Min	1st Pctl	25th Pctl	Median	75th Pctl	90th Pctl	99th Pctl	Max
Female	10,000	7	10.0	2.23	2	5	9	10	12	13	15	18
Male	10,000	7	10.0	2.23	2	5	8	10	11	13	15	18

Table 3.5.3. Simulations for Validation Set (N=30) by sex.

Group	N sims	10th Pctl	EV	Std Dev	Min	1st Pctl	25th Pctl	Median	75th Pctl	90th Pctl	99th Pctl	Max
Female	10,000	11	15.0	2.74	5	9	13	15	17	19	21	25
Male	10,000	11	15.0	2.74	5	9	13	15	17	19	21	25

[redacted]

[redacted]



[redacted]

Appendix C: Assignment of Subjects to Training and Validation Sets

1.1 Overview of Sample Assignment to the Training Set or Validation Set

1.1.1 Samples will be assigned to either the Training set or Validation Set using a pre-specified procedure designed to accomplish the following three objectives:

1.1.1.1 Ensure the Training and Validation sets are comparable across categories of interest (i.e., Conditions, Fitzpatrick Skin Type, Anatomical Region of Lesion, Age, and Sex), using a maximum Standard Mean Difference (SMD) <0.2.

The maximum SMD was chosen to assess comparablity because it is a widely accepted diagnostic measure of covariate balance in propensity score matched groups.[1] It can be applied in this setting to compare the frequency distributions of characteristics of interest between the Training and Validation sets. The use of a cut-off of 0.2 is based on Cohen's Effect[2] Size Index, and this theshold is a common choice for balance diagnostics in propensity score matching.

1.1.1.2 Minimize bias in assignment, and

1.1.1.3 Meet minimum enrollment targets for both the Training and Validation sets as specified in the study protocol.

1.1.2 To assign samples, 500 randomization assignment schedules will be generated. The first schedule that simultaneously meets the minimum enrollment targets for both the Training set and Validation set, and meets the requirement of a maximum Standard Mean Difference (SMD) <0.2 will be selected and used to assign samples to these sets.

[redacted]

[1] Austin PC. Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples. *Stat Med*. 2009;28(25):3083-3107. doi:10.1002/sim.3697

[2] Cohen J. *Statistical Power Analysis for the Behavioral Sciences* (2nd edn). Lawrence Erlbaum Associates Publishers: Hillsdale, NJ, 1988.

meeting pre-specified requirements. This schedule will be used to assign

[redacted]

[redacted]

The study protocol requires a minimum enrollment of (50) subjects, and assignment of 20 subjects to the Training Set and 30 subjects to the Validation Set. If more than 50 subjects are enrolled, excess subjects will be assigned to maintain the original ratio (40% of subjects assigned to the Training Set, 60% of subjects assigned to the Validation Set).

1.2.10 Validation of the Assignment Spreadsheet.

1.2.10.1 Prior to usage with real study data, the Assignment Spreadsheet will be tested and validated with simulated data. To generate a simulated data set, randomized categories will be assigned to hypothetical subjects using the probabilities expected in the population (Tables 1-5).

[redacted]