PI: **Sanchez, Gabriel**	Title: Noninvasive multiphoton imaging of subcellular structures with color contrast for rapid detection of skin cancers	
Received: 08/30/2020	Opportunity: RFA-CA-20-033	Council: 01/2021
Competition ID: FORMS-F	FOA Title: SBIR Phase IIB Bridge Awards to Accelerate the Development of Cancer-Relevant Technologies Toward Commercialization (R44 Clinical Trial Optional)	
2R44CA221591-03	Dual:	Accession Number: 4486504
IPF: 10033662	Organization: ENSPECTRA HEALTH, INC.	
Former Number: 2R44CA221591-02A1	Department:	
IRG/SRG: ZCA1 RPRB-6 (J2)	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 3: 1,968,512 Year 4: 1,212,820 Year 5: 815,870	Animals: N Humans: Y Clinical Trial: Y Current HS Code: 30 HESC: N HFT: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
[redacted]	ENSPECTRA HEALTH, INC.	PD/PI
[redacted]	ENSPECTRA HEALTH	Other Professional-Clinical Lead Scientist
[redacted]	Enspectra Health	Other Professional-Lead R&D Manager
[redacted]	ENSPECTRA HEALTH	Other Professional-Lead Clinical/Commercial Manager
[redacted]	Enspectra Health	Other Professional-Manufacturing and Operations Lead
[redacted]	Stanford University	Faculty
[redacted]	Stanford University	Faculty

Additions for Review

Supplemental Material	Supplemental Material: Video
Supplemental Material	Third Party Funds

OMB Number: 4040-0010
Expiration Date: 12/31/2022

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier
CA221591

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION **Organizational DUNS*:** [redacted]

Legal Name*: ENSPECTRA HEALTH, INC.
Department:
Division:
Street1*: ENSPECTRA HEALTH, INC.
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application
Prefix: Mr. First Name*: [redacted] Middle Name: Last Name*: [redacted] Suffix:
Position/Title: President/COO
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: [redacted] Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

7. TYPE OF APPLICANT* R: Small Business

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*
❍ New ❍ Resubmission
● Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Noninvasive multiphoton imaging of subcellular structures with color contrast for rapid detection of skin cancers

12. PROPOSED PROJECT
Start Date* Ending Date*
04/01/2021 03/31/2024

13. CONGRESSIONAL DISTRICTS OF APPLICANT
CA-018

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: [redacted] Middle Name: Last Name*: [redacted] Suffix: [redacted]
Position/Title: Co-founder and CEO
Organization Name*: ENSPECTRA HEALTH, INC.
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested* [redacted]
b. Total Non-Federal Funds* [redacted]
c. Total Federal & Non-Federal Funds* [redacted]
d. Estimated Program Income* [redacted]

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ❍ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

❍ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Mr. First Name*: [redacted] Middle Name: Last Name*: [redacted] Suffix:
Position/Title*: President/COO
Organization Name*: Enspectra Health, Inc.
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: [redacted] Email*: [redacted]

Signature of Authorized Representative*

[redacted]

Date Signed*

08/30/2020

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:COVERLETTER_EnSpectra.pdf

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 12/31/2022

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: ENSPECTRA HEALTH, INC.
Duns Number: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: [redacted]

Additional Location(s) File Name:

Funding Opportunity Number: RFA-CA-20-033. Received Date: 2020-08-30T20:48:47.000-04:00

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ○ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ● No

If YES, check appropriate exemption number: __ 1 __ 2 __ 3 __ 4 __ 5 __ 6 __ 7 ___ 8

If NO, is the IRB review Pending? ● **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number [redacted]

2. Are Vertebrate Animals Used?* ○ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	PROJECTSUMMARYABSTRACT.pdf
8. Project Narrative*	PROJECTNARRATIVE.pdf
9. Bibliography & References Cited	REFERENCES.pdf
10.Facilities & Other Resources	EH_FACILITIES_-_Sobrato.pdf
11.Equipment	EQUIPMENT_-_EnSpectra.pdf
12. Other Attachments	SBC_000418136.pdf Video_Attachment_EnSpectra_Health.pdf

Funding Opportunity Number: RFA-CA-20-033. Received Date: 2020-08-30T20:48:47.000-04:00

Nonmelanoma skin cancer (NMSC) represents the most common form of cancer in the human body. The method for diagnosing and treating NMSCs requires a skin biopsy that is processed and stained for analysis on a standard optical microscope. This process is painful for patients, and the invasiveness of biopsy introduces a delay into NMSC detection, which contributes to patient morbidity and adds substantial cost to the healthcare system. EnSpectra Health, Inc (Enspectra) aims to address the unmet clinical need for a better method to detect NMSCs earlier. This Phase IIB application builds on the progress of awarded Phase I and Phase II projects (NCI, 2R44CA221591-02A1). In Phase I of this grant, EnSpectra demonstrated the feasibility of a portable, skin-imaging microscope for human clinical studies (Aim 1), developed software for data acquisition, image processing, and improved user interface (Aim 2), and demonstrated in 5 human subjects with basal cell carcinoma (BCC) the ability to detect pathologic features of BCC (Aim 3). EnSpectra has created the first fiber coupled multiphoton microscopy (MPM) system for *in vivo* imaging of skin cellular anatomy. In Phase II of this grant, EnSpectra advanced the development of its portable, skin-imaging microscope (Aim 1), developed a library of slide images of targeted skin diseases comparing MPM to traditional histology (Aim 2), and is on track to complete *in vivo* human performance testing (Aim 3) prior to the Phase II end date. The completion of the Phase II aims will enable EnSpectra to build clinical evidence in to support a 510(k) submission for United States Food & Drug Administration (FDA) clearance. In this Phase IIB proposal EnSpectra will accomplish two aims: Aim 1) Develop and manufacture 8 portable, skin-imaging microscopes for commercial readiness (12 months), Aim 2) Conduct clinical trial to support reimbursement body of literature (24 months).

EnSpectra Health, Inc. is continuing to develop its noninvasive cellular imaging system to aid in the early detection of nonmelanoma skin cancers, the most common form of malignancy. We will build and test several systems and execute a multi-site study to collect clinical evidence to secure reimbursement coverage for our commercial product as we bring our technology into clinical practice. Our innovation will provide point-of-care clinicians with unprecedented real-time access to cellular histology, ensuring potential cancers are discovered as soon as possible.

FACILITIES

The EnSpectra team has access to outstanding facility resources as the result of its relationship with the Fogarty Institute for Innovation (FII). FII is a medical device incubator whose primary role is the support of early stage companies. FII provides an environment of collaboration and sharing of expertise between companies-in-residence, which is fostered by large, open shared office and laboratory spaces. Residing in a new state of the art facility adjacent to El Camino Hospital, the FII fosters collaboration with nearby physicians and provides access to patient populations for clinical studies. FII hosts frequent seminars led by cross-disciplinary experts in the medical device industry. In addition, world-class entrepreneurs and physicians with deep experience in medical device commercialization participate actively in the Fogarty Institute and volunteer their time to advise and make introductions for the companies-in-residence.

In addition to the collaborative environment, FII also provides ample physical space and resources. The primary equipment and facilities are already in place to complete the proposed project, as evidenced by EnSpectra's ability to build and sell commercial research microscopes for imaging muscle and complete the Aims of Phase I and Phase II for imaging skin. Additionally, the FII has recently entered into a long-term lease with El Camino Hospital to occupy an entire floor (nearly 25,000 square feet) of a 300,000 square foot facility (The Sobrato Pavilion) which will allow it to support its existing and future companies-in-residence. This facility is ready for occupancy as of the time of this submission and EnSpectra plans to move its operations to the new facility in September of 2020. The new facility represents a 250% increase in available space from the previous FII accommodations housed in a single contiguous floor space. By residing in this space, EnSpectra will have ample facilities support for the full term of the proposed project (see Figure 1 for floor plan).

Laboratory: EnSpectra Health resides at FII, which is located *adjacent* to El Camino Hospital in the newly opened Sobrato Pavilion in Mountain View, California. This new facility provides ample space for the Fogarty staff, expanded medical device R&D lab capabilities, a machine shop, and manufacturing facilities for the companies-in-residence. The facility is approximately 25,000 square feet and brings together the entire FII operation under one roof, ultimately replacing the previous facilities.

FII currently hosts approximately 10 companies-in-residence. EnSpectra will be able to leverage an expanded common laboratory space (over 6,500 ft^2 of useable storage and work space) and will also have its own dedicated lab, manufacturing, and clinical trial space that will be for EnSpectra exclusive use during the project. Relevant shared and exclusively owned equipment is described in the "Equipment" section. Part of the shared laboratory includes a large wet lab for conducting *in vitro* or *ex vivo* studies. EnSpectra also has exclusive access to several bays of laboratory space within Sobrato.

Animal: Not applicable. Due to the low risk nature of the microscope and the proof-of-concept already completed in human skin and muscle tissue, animal studies or facilities are not required to validate the proposed technology.

Computer: Each EnSpectra employee has a laptop or personal computer for engineering design, literature review, data analysis, purchases, sales, communication, and composition. There are several additional computers dedicated to the current skin-imaging and Slide Atlas prototype, as well as a robust data server for the storage and analysis of data collected by the skin-imaging systems. EnSpectra is equipped with multiple software packages for engineering

design such as Solidworks, Matlab, Adobe Creative Suite, and Zemax. We have recently relaunched a new Quality Management System for tracking the development and risk management of our technology in accordance with regulatory standards, and have implement electronic signature procedures (via a FDA part 11 compliant module provided by DocuSign).

The corporate standard for Office productivity tools is MicroSoft365 which provides e-mail (Outlook), Office tools (Excel/Word/Powerpoint), file storage and collaboration (Sharepoint), and employee communication and collaboration (Teams). The environment is hosted in the cloud, allowing for secure access by EnSpectra employees via any internet connection.

Within Sobrato, FII hosts wired and wireless internet for all companies in residence and provides printing, mail and other office productivity services. FII also provides teleconference-specific phones in all of its nine conference rooms. In addition, EnSpectra has implemented IP based telephony solutions for employee, consultant, and partner interaction (Grasshopper and UberConference/Zoom). Enspectra also has its own dedicated projector for group meetings and presentations.

Office: Similar to the laboratory, EnSpectra benefits from a large shared office space within the dedicated FII Sobrato office space (including nearly 5000 ft^2 of meeting rooms, kitchen, indoor and outdoor eating areas, and office equipment areas), which is directly adjacent to the laboratory. The five employees at EnSpectra, including the PI, shares exclusive access to a large office cubicle within the new office space (over 400 ft^2). Within the shared office space, FII maintains multiple color printers, scanners, photocopiers, and paper shredding bins housed in a dedicated printer/copier room and placed throughout the office space. FII also provides basic office supplies, such as envelopes, tape, paper, staplers, and writing utensils. There are nine shared conference rooms of varying sizes in close proximity to EnSpectra's work space with a large screen TVs for projection, seating for up to 30 people, multiple white boards, and phones for teleconferences. These conference rooms are professionally designed and are ideal for client and investor presentations. The FII office space is staffed during business hours by FII administrative personnel to manage the space, accept packages, greet and direct visitors, and provide administrative support to companies-in-residence. There is also a nearly 2,300 square foot shared café and kitchen with indoor and outdoor seating areas for natural collaboration between employees of the various resident companies and the FII mentors. The facility also boasts of ample storage space for materials, equipment and other supplies to support the proposed project.

Other: There is a 1,500 square foot machine shop directly adjacent to the laboratory, which includes a lathe, drill press, mill, vertical band saw, horizontal band saw, and FDM 3D printer. These machines have been used extensively in prototyping and building the research muscle imaging microscopes, the skin-imaging prototype used to complete the Phase I amd Phase II Aims, and will be heavily used for the proposed project described here. FII also has available space earmarked for an ISO 7 compliant clean room, which EnSpectra may use to complete custom optical assemblies. FII provides regular cleaning, garbage disposal, and biohazard disposal throughout their office and laboratory spaces.

Of particular note, the Sobrato facility will enable EnSpectra to maintain its own patient exam room for conducting human subject research in-house as well as a dedicated EnSpectra study waiting room. EnSpectra has previously conducted two IRB-approved studies using human subjects using similar infrastructure provided by FII.

Figure 1. FII Sobrato facility floor plan

EQUIPMENT

All listed equipment is available at our current location in the Fogarty Institute for Innovation (FII), Mountain View CA. Equipment owned by FII is indicated parenthetically. EnSpectra already owns most of the specialized equipment and tooling necessary to prototype and build its products.

Optical prototyping:

- 780 nm femtosecond pulsed fiber laser (this is our table top prototyping laser we use for bench testing and is owned by EnSpectra)
- 980, 780, 670, 660, 532, 405 nm diode Lasers
- Various optical filters, lenses, mirrors, ½ waveplates, ¼ waveplates, dichroics,
- Assorted optomechanics: breadboards, posts, XYZ stages, kinematic mirror mounts, fiber launch, laser safety goggles
- Programmable Motorized High Precision XY stage
- 780 and 1030 nm optical isolators
- Various multimode fibers
- Various liquid light guide fibers
- 780 and 1030 single-mode fibers
- Various photomultiplier tubes
- Various transimpedence amplifiers
- Optical Targets –

Ronchi Rulings, Air Force Targets, Fluorescent Bead Targets, Fluorescent Slides

Mechanical prototyping

- Fully outfitted machine shop (FII)
- FDM uPrint SE Plus 3D Printer (FII)
- 2 axes CNC vertical knee mill (FII)
- Hardinge manual lathe (FII)
- Drill press (FII)
- Vertical band saw (FII)
- Horizontal band saw (FII)
- Disk grinders (FII)
- Belt sanders (FII)
- Sand blasting cabinet (FII)
- Basic hand tools including drill, dremel, taps, screwdrivers, files, micrometers, calipers
- EnSpectra Health owns its own parallels, cutters, drills, indicators, rotary tables, and other machine shop necessities
- Raw materials: metal and plastic sheet, rods, tubing, wires etc.

Electrical Prototyping

- 4 channel bench oscilloscope
- 2 channel portable oscilloscope
- function generator
- +5/+-15 3 amp bench power supply
- 2 multimeters
- 2 soldering stations
- Full resistor and capacitor cabinets
- Heat guns
- Various connectors, wires, breadboards, motors, chips, etc.

Specialty measurement equipment

- 1030 nm autocorrelator
- Beam profiler
- Power meter

- IR camera
- Position sensitive detectors

Assembly equipment

- 2 stereomicroscopes (FII)
- 2 UV guns
- Curing oven
- Vacuum pump
- Ultrasonic cleaner
- Small batch autoclave
- Large batch autoclave (FII)

Other Equipment (all is FII owned)

- Cabinets for flammable chemicals
- Freezers – surgical discards
- Refrigerators – surgical discards
- Eye wash stations
- Fire extinguishers
- Multiple sinks

SBIR · STTR
America's Seed Fund™
POWERED BY SBA

SBIR.gov SBC Registration

SBC Control ID:	[redacted]		
Company Name:	Enspectra Health, Inc.		
Address:	[redacted]		
City:	[redacted]		
State:	[redacted]	**Zip:**	[redacted]
EIN (TIN):	[redacted]	**DUNS:**	[redacted]
Company URL:			

Number of Employees:	1
Is this SBC majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?	No
What percentage (%) of the SBC is majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?	0.00%

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Gabriel Middle Name Last Name*: Sanchez Suffix: Ph.D

Position/Title*: [redacted]
Organization Name*: [redacted]
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: PHD Degree Year: [redacted]

Attach Biographical Sketch*: File Name: Biosketch_-_GNS.pdf

Attach Current & Pending Support: File Name:

Funding Opportunity Number: RFA-CA-20-033 . Received Date: 2020-08-30T20:48:47.000-04:00

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: [redacted] Middle Name Last Name*: [redacted] Suffix: [redacted]

Position/Title*: [redacted]
Organization Name*:
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*:
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login:

Project Role*: Other Professional Other Project Role Category: [redacted]

Degree Type: PHD Degree Year: [redacted]

Attach Biographical Sketch*: File Name: [redacted]

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Mr. First Name*: [redacted] Middle Name Last Name*: [redacted] Suffix:

Position/Title*: [redacted]
Organization Name*:
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*:
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login:

Project Role*: Other Professional Other Project Role Category: Lead R&D Manager

Degree Type: MS Degree Year: [redacted]

Attach Biographical Sketch*: File Name: [redacted]

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Mr. First Name*: [redacted] Middle Name Last Name*: [redacted] Suffix:

Position/Title*: [redacted]
Organization Name*:
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*:
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login:

Project Role*: Other Professional Other Project Role Category: [redacted]

Degree Type: MBA Degree Year:

Attach Biographical Sketch*: File Name: [redacted]

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Mr. First Name*: [redacted] Middle Name Last Name*: [redacted] Suffix:

Position/Title*: [redacted]
Organization Name*:
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*:
Province:
Country*: [redacted]
Zip / Postal Code*:

Phone Number*: [redacted] Fax Number: [redacted]

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: Other Professional Other Project Role Category: [redacted]

Degree Type: BS Degree Year: [redacted]

Attach Biographical Sketch*: File Name: [redacted]

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Prof. First Name*: [redacted] Middle Name: [redacted] Last Name*: [redacted] Suffix:

Position/Title*: [redacted]
Organization Name*: [redacted]
Department:
Division:
Street1*: [redacted]
Street2:
City*:
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: [redacted]

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: Faculty Other Project Role Category:

Degree Type: PHD,MS,BS Degree Year: [redacted]

Attach Biographical Sketch*: File Name: [redacted]

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Prof. First Name*: [redacted] Middle Name: [redacted] Last Name*: [redacted] Suffix: [redacted]

Position/Title*: [redacted]
Organization Name*: [redacted]
Department:
Division:
Street1*: [redacted]
Street2:
City*:
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: Faculty Other Project Role Category:

Degree Type: PHD,MA,AB Degree Year: [redacted]

Attach Biographical Sketch*: File Name: [redacted]

Attach Current & Pending Support: File Name:

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: ENSPECTRA HEALTH, INC.

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	[redacted]					PD/PI							
2.						Lead Scientist							
3.						Product Development Lead							
4.						Clinical Lead							
5.						Manufacturing Lead							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-CA-20-033 . Received Date: 2020-08-30T20:48:47.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: ENSPECTRA HEALTH, INC.

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
1 . Ultrafast Near IR Laser with SHG module (Qty - 8)	[redacted]
2 . Voice Coil Stage (Qty - 8)	[redacted]
Total funds requested for all equipment listed in the attached file	
Total Equipment	[redacted]

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	[redacted]
2. Foreign Travel Costs	
Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	[redacted]

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: ENSPECTRA HEALTH, INC.

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	[redacted]
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	[redacted]
7. Alterations and Renovations	
8 . Professional Manufacturing Services	[redacted]
9 . Clinical Trial Expenses	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name: Budget_EH_PhaseIIB_Aug2020_Final.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT13194452 Funding Opportunity Number: RFA-CA-20-033 . Received Date: 2020-08-30T20:48:47.000-04:00

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: ENSPECTRA HEALTH, INC.

Start Date*: 04-01-2022 **End Date*:** 03-31-2023 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	[redacted]					PD/PI							
2.						Lead Scientist							
3.						Product Development Lead							
4.						Clinical Lead							
5.						Manufacturing Lead							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-CA-20-033 . Received Date: 2020-08-30T20:48:47.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: ENSPECTRA HEALTH, INC.

Start Date*: 04-01-2022 **End Date*:** 03-31-2023 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	[redacted]

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	[redacted]
2. Foreign Travel Costs	
Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	[redacted]

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: ENSPECTRA HEALTH, INC.

Start Date*: 04-01-2022 **End Date*:** 03-31-2023 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	[redacted]
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	[redacted]
7. Alterations and Renovations	
8 . Professional Manufacturing Services	[redacted]
9 . Clinical Trial Expenses	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name: Budget_EH_PhaseIIB_Aug2020_Final.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT13194452 Funding Opportunity Number: RFA-CA-20-033 . Received Date: 2020-08-30T20:48:47.000-04:00

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: ENSPECTRA HEALTH, INC.

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	[redacted]					PD/PI							
2.						Lead Scientist							
3.						Product Development Lead							
4.						Clinical Lead							
5.						Manufacturing Lead							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-CA-20-033 . Received Date: 2020-08-30T20:48:47.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: ENSPECTRA HEALTH, INC.

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	[redacted]

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	[redacted]
2. Foreign Travel Costs	
Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	[redacted]

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT13194452 Funding Opportunity Number: RFA-CA-20-033 . Received Date: 2020-08-30T20:48:47.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: ENSPECTRA HEALTH, INC.

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	[redacted]
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	[redacted]
7. Alterations and Renovations	
8 . Professional Manufacturing Services	[redacted]
9 . Clinical Trial Expenses	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name: Budget_EH_PhaseIIB_Aug2020_Final.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification (Research & Related Budget)

"Noninvasive multiphoton imaging of subcellular structures with color contrast for rapid detection of skin cancers"

Introduction

This budget outlines the costs necessary to complete the proposed specific aims:

Aim 1) Develop and manufacture 8 portable, skin-imaging microscopes for clinical trial execution and commercial readiness.
Aim 2) Conduct clinical trial(s) to support reimbursement body of literature.

We request total funding support of [redacted] in direct and indirect costs over a 3-year period.

A Senior/Key Personnel – EnSpectra Health, Inc. ([redacted]).

Name	Title	Annual Salary	Project Responsibility	Time (mos)	Rate ($/mo)	Total	Year 1	Year 2	Year 3
[redacted]	CEO, PI	[redacted]	Management of overall project	18	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	Manager, Clinical and Medical Affairs	[redacted]	Clinical planning and execution	22	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	Manager, Engineering	[redacted]	Leader of product development	22	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	Chief Commercial Officer	[redacted]	Management and execution of clinical, regulatory, commercial	14	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	President/COO	[redacted]	Mgmt and execution of manufacturing, supply chain, compliance	14	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	co-founder	$0	Mentorship: FDA dynamics, product design and Software	4	$0	$0			
[redacted]	co-founder	$0	Mentorship: Corporate strategy product design and Optics	4	$0	$0			
					Total salary and wages	[redacted]	[redacted]	[redacted]	[redacted]

In this Phase, we are dedicating our efforts to building systems to collect clinical evidence and to conducting a clinical trial that will support commercial reimbursement of the EnSpectra system. As this effort is essential to our overall commercial success and requires a large body of evidence, we will spend proportionally more time on this in Phase IIb than we did in our Phase II. Also, because the activities we are proposing in this Phase IIb are directly aligned with the overall company objective to move toward a commercial launch of our product, all of our full-time employees will dedicate a significant portion of their time to this project.

A.1 [redacted], Principal Investigator [redacted], base salary [redacted]/year). [redacted] will commit 50% of his time to lead and manage this project. [redacted] will oversee activities for both Specific Aims.

[redacted] has been CEO of EnSpectra Health (formerly Zebra Medical Technologies) for 6 years and has successfully executed the company's vision to enable *in vivo* imaging of cellular anatomy. Under his leadership, the Phase I application successfully yielded the world's smallest and only fiber coupled handheld MPM system for live skin imaging. He has continued to develop the clinical and commercial opportunity for earlier detection of skin cancers using EnSpectra's proprietary technology. [redacted] has consistently attracted substantial interest from venture capitalists in the medical device and digital health industries in a very difficult investment environment, and has successfully completed several fund-raising activities, including the recent closing of a Series A financing in 2019. [redacted] has experience with conducting human investigations, including a study within the Fogarty Institute as part of the successful Phase I of this grant proposal. As a member of the Fogarty Institute, he is enmeshed in a vast network of medical practitioner and medical device industry experts and regularly participates in interdisciplinary discussions about regulatory and reimbursement affairs and effective strategies for commercializing medical technologies. His broad experience continues to grow and ranges from managing complex R&D projects to business development, fundraising, and clinical planning. In the Phase IIb of this proposal, [redacted] will guide the vision and development of an advanced and scalable version of the EnSpectra system that will undergo testing in a clinical setting to build data for reimbursement coverage and commercial launch. His breadth of experience and previous successes make him an ideal leader for this project.

[redacted] holds a B.S. in mechanical engineering from MIT, as well as an M.S. and Ph.D. in mechanical engineering from Stanford University. He has extensive design and fabrication experience of optical, optomechanical, and electromechanical devices. [redacted] spearheaded the Phase I efforts of this proposal that lead to the creation of the world's first handheld, battery powered, MPM system. As CEO of EnSpectra Health, he has led rapid advancement and development that has attracted the interest of investors and clinicians for a novel application in early skin cancer detection. He has served as a co-founder and consultant to multiple medical device startups ranging from laparoscopic to ophthalmic devices. In 2011, [redacted] attended the Summer Institute for Entrepreneurship at the Stanford Graduate School of Business. [redacted]

[redacted] **– 22 months; base salary [redacted]/year).** [redacted] is the Manager of Clinical and Medical Affairs at EnSpectra Health and will dedicate most of her 60% contribution to the completion of Specific Aim 2. Over the past 4 years, [redacted] has worked hand-in-hand at EnSpectra Health with a talented team of medical device professionals to develop a deep understanding of the technology's positioning amidst complex clinical and reimbursement dynamics. In our successful Phase I and Phase II projects, [redacted] successfully planned and executed clinical testing, data analysis, quality control, and publication strategy. With her experience, [redacted] is well positioned to advance the clinical trial and publication activities of this Phase IIb proposal.

[redacted] holds a B.S. from Rice University in bioengineering and M.S. and Ph.D. degrees in bioengineering from Stanford University. [redacted] has extensive animal and human research experience with an excellent record of developing novel methods and experimental techniques for optical bioinstrumentation. [redacted] work is published in top tier journals such as Nature Biotechnology, Nature Methods, and Science Translational Medicine. [redacted] will assist in product and clinical study design for the reimbursement trial(s) and lead our efforts in experimental design, human subject testing, data analysis and publication of results.

A.3 [redacted] – Engineering and Product Development Manager. ([redacted] – 22 months; base salary [redacted]/year). [redacted] is the Manager of Engineering at EnSpectra Health and will dedicate most of his 62% contribution to the completion of Specific Aim 1. [redacted] has been leading the technical design efforts at EnSpectra since May 2019 and has participated in the advancement of the first-generation prototype system built in our successful Phase I proposal. In our successful Phase II proposal, he has been responsible for advancing our technical design to produce more reliable and scalable systems, and has led a team of engineers and technicians to build and test a second generation device used in Specific Aims 2 and 3 of that project. [redacted] has over a decade of experience in startup and commercial medical device companies and is well practiced in research and development activities leading to clinical data collection, product clearance and commercialization. With his prior experience both at EnSpectra and with other companies, [redacted] will be a central figure to drive the product development activities of this Phase IIb proposal.

[redacted] holds a B.S. from University of Mumbai in control systems and instrumentation and an M.S. degree in bioengineering from Drexel University. [redacted] work is published in journals such as Applied Optics and in the Society for Biomaterials. He is also a named inventor on several patents. [redacted] will lead product design and product development efforts for the manufacture and testing of the devices/systems to be used in the proposed reimbursement trial(s) and will participate in the experimental design, human subject testing, data analysis and publication of results.

A.4 [redacted] – Clinical and Reimbursement Leader. ([redacted] – 14 months; base salary [redacted]/year). [redacted] is the Chief Commercial Officer at EnSpectra Health and will dedicate most of his 40% contribution to the completion of Specific Aim 2. [redacted] has been working for EnSpectra since July 2019 and has led the Regulatory, Clinical, and Commercial strategy and execution of the company for the past year. In addition to leading the analysis of the market opportunity and developing the regulatory and clinical strategy, [redacted] has collected valuable input from our target customers which has been critical to the advancement of the prototype system from our successful Phase I proposal into a second generation device to be used in Specific Aims 2 and 3 of our nearly completed Phase II proposal. In our successful Phase II proposal, [redacted] has overseen user requirements collection, clinical evidence gathering, reimbursement analysis, clinical data analysis, and publications. With his prior experience, [redacted] is uniquely positioned to support the clinical trial and publication activities of this Phase IIb proposal.

[redacted] holds a B.A. in Economics from University of California, Los Angeles and an MBA from the Wharton School at the University of Pennsylvania. [redacted] has 30 years of experience in both hands on and leadership roles across a variety of health care businesses including pharmaceutical, medical device, diagnostics, and therapeutics at companies such as Merck, Johnson & Johnson, and Roche Molecular in addition to startup medical device companies. He has experience in sales and marketing, business development, corporate finance, clinical study design and execution, and development of pricing and reimbursement strategies. He has led successful product launches in both startups and large companies.

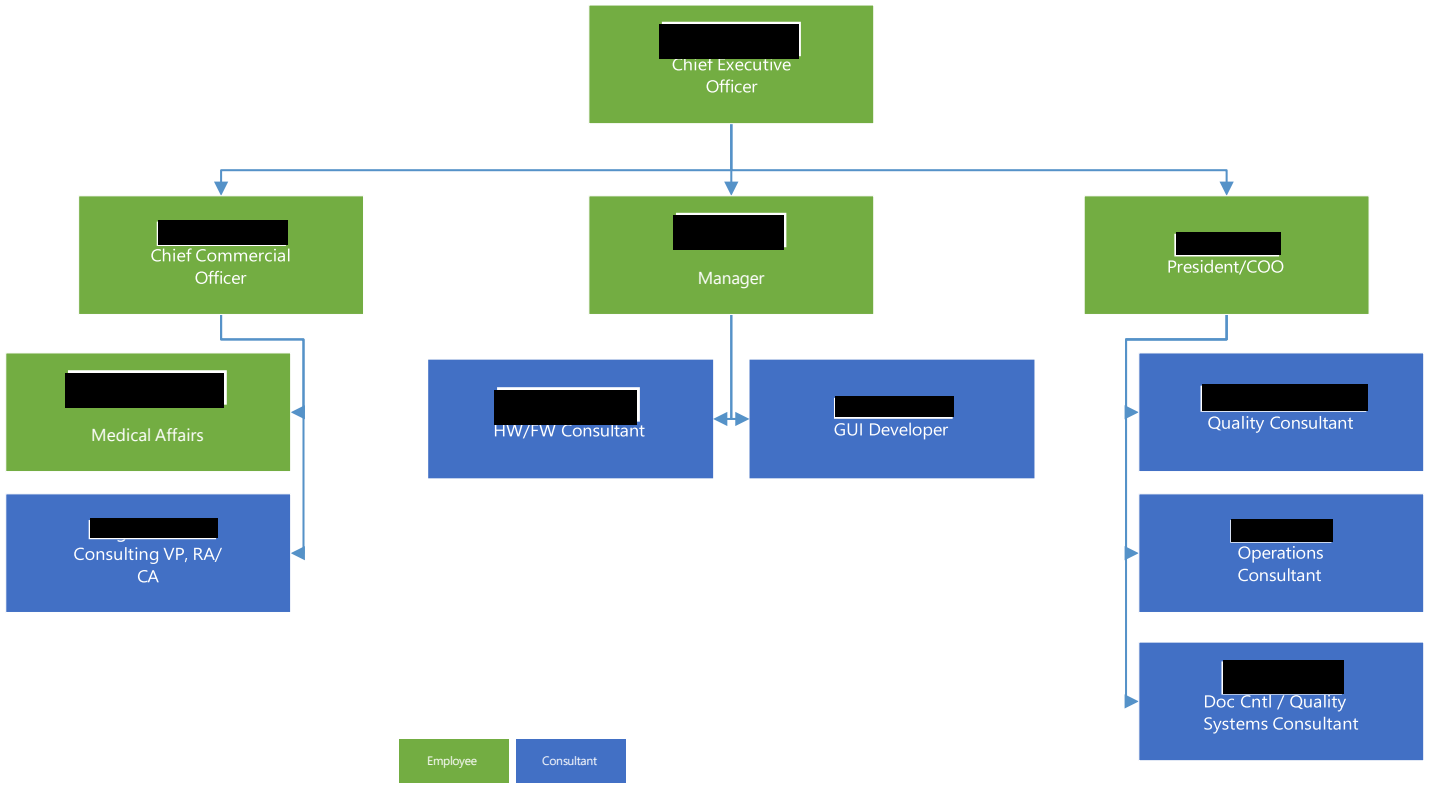
A.5 [redacted] – Manufacturing and Infrastructure Leader. ([redacted] – 14 months; base salary [redacted]/year). [redacted] is the President and Chief Operating Officer at EnSpectra Health and will dedicate most of his 40% contribution to supporting Specific Aim 1. [redacted] has been working for EnSpectra since April 2019 and has supported the product development strategy and execution as well as the corporate infrastructure of the company for the past 18 months. He will be working closely with the product development team to deliver the systems that will be used in the proposed clinical trial(s), and will lead a team of internal and external resources to development and execute the required manufacturing, supply chain, and quality processes. With his prior experience, [redacted] is uniquely positioned to support both the product development and the clinical trial activities of this Phase IIb proposal.

[redacted] holds a B.S. in Pre-Professional Studies from University of Notre Dame. He has nearly 20 years of management experience in medical device operations, manufacturing, infrastructure and executive management. In addition to his operations responsibilities he has overseen regulatory and clinical strategy, product development, intellectual property, general and administrative functions, customer service and product distribution. He has supported successful product launches and manufacturing scale up activities in several startup device companies that have been acquired by large medical companies such as Medtronic and Stryker Corporation. Prior to his medical device experience, [redacted] spent over 10 years developing and implementing technology solutions (software and hardware) for a variety of small, medium, and large companies across multiple industries.

A.6 [redacted] – Cofounder and Mentor. ([redacted] – 4 months; base salary [redacted]/year). [redacted] is a cofounder of Enspectra Health and volunteers his time to mentor and advise the team. [redacted] is a co-founder in another medical device company that recently secured a De Novo regulatory clearance from the FDA. As such, he has recent and relevant insights into the regulatory dynamics at the FDA and can advise EnSpectra to increase the likelihood of success. [redacted] is the director of the Stanford Neuromuscular Biomechanics Laboratory and is a world-renowned expert in the development of open source software tools, such as OpenSim and Simbody, for computer simulation of human biomechanics. [redacted] received a Ph.D. from Stanford University in 1990 and joined the faculty at Northwestern University from 1991-1999. He returned to Stanford in 1999, where he currently is the James H. Clark Professor of Bioengineering, Mechanical Engineering, and Orthopaedic Surgery. [redacted] has previously founded several successful companies and is currently a co-founder in three early stage startups including EnSpectra.

A.7 [redacted] – Cofounder and Mentor. ([redacted] – 4 months; base salary [redacted]/year). [redacted] is a cofounder of EnSpectra Health and volunteers his time to mentor and advise the team in matters of corporate strategy, product design, and optical design. [redacted] is a preeminent professor of biology and applied physics at Stanford University who has pioneered microendoscopy for *in vivo* imaging in both animals and humans. [redacted] is an expert in the custom design of optical instrumentation and multiphoton imaging techniques. After receiving a Ph.D. from Princeton University, [redacted] developed the first one and two-photon fiber-based imaging systems to study neurological behavior in live rodents. [redacted] research at Stanford has spawned multiple scientific breakthroughs, including a successful start-up that he co-founded to provide neuroscience researchers with a user-friendly end-to-end solution for *in vivo* brain imaging in freely behaving rodents.

Company Org Chart



B Other Personnel. None.

C Equipment ([redacted]).

Equipment	Vendor and Description	Unit cost	Units	Total	Year 1	Year 2	Year 3
Ultrafast near IR laser with SHG module	Toptica fiber laser to serve as light source for skin imaging prototype (includes Miniature PPLN 1560nm to 780 nm converter)	[redacted]	8	[redacted]	[redacted]		
Voice Coil Stage	V-900K047 PIMag™ VC Vertical Linear Actuator, 1.5mm travel, 2m cable, adapted electronics for direct use with C-413	[redacted]	8	[redacted]	[redacted]		
		Category total cost		[redacted]	[redacted]		

We are requesting [redacted] for portable ultrafast near IR fiber lasers with included miniature SHG conversion module and voice coil stage. This system is the core engine of our current system and delivers 1560nm light through a fiber optic to a miniature PPLN based converter that emits 780nm light to excite endogenous signals in the skin. We found 780nm to be an ideal wavelength to excite the multiphoton signals we need from skin tissues. This laser has a small footprint and an optical fiber output which are essential features for our clinical prototype to image skin cancers. Quotes for this equipment have been attached in the grant submission portal as Other Attachments.

- The laser [redacted] cost is based on a purchase of eight units. This is not a sole source item as there are other manufacturers of analogous products, but we have a previous history with [redacted]. We co-developed this miniature combination to create a one of a kind ultrafast system. This device meets all necessary certifications to begin our work. The price is not derived from a competitive bid; our preference is to work with this manufacturer given prior experience working with them.

- [redacted] quoted the voice coil stage [redacted]. This voice coil controls the axial scan by translating a lens within the handheld microscope along its optical axis. This is not a sole source vendor. We have all certifications and agreements necessary to begin work with these components immediately. This was not a competitive bid; we chose [redacted] due to our familiarity with their products from our previous technology.

D Travel ($123,000).

Travel	Unit cost	Units	Total	Year 1	Year 2	Year 3
Clinical Site initiation, support, monitoring visits	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Component Supplier/Vendor visits/audits						
Contract Manufacturer visits					[redacted]	
Category total cost			[redacted]	[redacted]	[redacted]	[redacted]

We have allocated [redacted] for travel to the participating clinical sites in our clinical trial and for trips to our manufacturing and supply chain partners to support device development and manufacturing. For the clinical trips, we estimate this amount will be sufficient for one or two members of

our team to travel to sites for the initial site qualification and up to three on-site visits to manage and promote subject recruitment and to support subject visits. These trips will require airfare, a one-night stay at a hotel, local transportation, and associated meals.

For the supplier visits, the estimates represent two or three trips per year for one to two team members. These trips will require airfare, a one- or two-night stay at a hotel, local transportation, and associated meals.

E Participant Support Costs. None.

F Other Direct Costs. We have direct costs for materials and supplies (F.1), consultant services (F.3), facility rental (F.6), professional R&D/Mfg services (F.8), and clinical trial expenses (F.9).

F.1 Materials and Supplies: ($).

Materials and Supplies	Vendor and Description	Unit Cost	Units	Total Cost	Year 1	Year 2	Year 3
Controllers	- voice coil controller, Mirrorcle - MEMS driver						
Integrated Printed Circuit Board	- custom PCBA						
AC/DC Converter and Isolation Transformer	electrical power components, specific part numbers to be determined						
Display Unit	- PC, software license, cables						
Multi-Pixel Photon Counters	- MPPC solid state photo multiplier (quantity 3 per system)						
Base Station Optical Components	(lenses, mirrors, filters)						
Base Station Machined Parts	(collection box)						
Base Station Enclosure	or TBD - molded or 3D printed, 2nd ops						
Wand Optical Parts	(lenses, mirrors, filters, fiber, light guide)						
Wand Machined Parts	(mounts, holders, spacers)						
Machined Fixtures/Tools	(fixtures/tools required for assembly)				672		
Wand Custom Housing	or TBD - molded or 3D printed, 2nd ops						
MEMS Scanner	- mirror scanner						
Disposable Cap	– molded silicone cap to protect lens/objective						

Materials and Supplies	Vendor and Description	Unit Cost	Units	Total Cost	Year 1	Year 2	Year 3
UV Curing Station	[redacted] UV Curing station with Radiometer	[redacted]	[redacted]	[redacted]	[redacted]		
Material/Quarantine cages	Storage and organization of product parts, components, sub-assemblies	[redacted]	[redacted]	[redacted]	[redacted]		
Clinical/Recruitment	Ads, printed materials, binders, distribution	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
				Category total cost	[redacted]	[redacted]	[redacted]

We have requested [redacted] for materials and supplies necessary to complete our Specific Aims. We plan to build 8 functional systems. We will need six for data collection at the clinical sites, while the remaining two are for verification and validation testing. All costs are based on previous purchases, quotes, or online pricing for standard components.

- Controllers including the voice coil controller and MEMS mirror driver is @[redacted]. These controllers drive the main scanning actuators in our device. The voice coil controller is supplied by [redacted], who supplies our voice coil stage. The MEMS driver is supplied by [redacted] who supplies our actuated MEMS mirror.
- [redacted] quote for the Integrated Printed Circuit Board is @[redacted]. It is a custom designed board with onboard analog and digital processing, which allows our system to reconstruct images using FPGA architecture while being controlled using an off the shelf programmable Microcontroller unit. This integrated PCB allows our system to construct images and display them in real time frame rates, 4 times faster than the previous architecture which was based on the USB National Instruments DAQ board.
- AC/DC Converter and Isolation Transformer from [redacted] is @[redacted].
- The display unit quoted by [redacted] is @[redacted], which is their standard offering for a medical grade tablet that conforms to use environments in clinical settings. Software licenses and cables are additive costs.
- [redacted] quote for Multi-Pixel Photon Counters is @[redacted] which is less than half the price of previously used PMTs. The Multi-Pixel Photon Counter (MPPC), also known as silicon photomultiplier (SiPM), are solid state photomultipliers that have high internal gain which enable single photon detection, with a low dark count, high photon detection efficiency, excellent timing resolution, low bias voltage operation. We need one per channel, therefore a total of 3 are needed per system for a cost of @[redacted].
- [redacted] quotes for base station optical components total @[redacted]. This item estimates the cost of the lenses, optical fibers, mirrors, filters, sensors, etc. necessary to construct our portable dermatology device. These are not sole source vendors. We have all certifications and agreements necessary to begin work with these components immediately. This was not a competitive bid; we chose these distributors and OEMs due to our familiarity with their products from our previous technology.
- [redacted] quotes for machined components in the base station total @[redacted]. This serves as the chassis for the briefcase containing the laser, sensors, and processing electronics. This is not a sole source vendor. We have all certifications and agreements necessary to begin work with these components immediately. This was not a competitive bid; we chose [redacted] due to our familiarity with their products from our previous technology.
- The Base Station enclosure will be molded or 3-D printed with subsequent machining. Potential supplier is [redacted]. Estimate @[redacted] is based on prior experience working with supplier ProtoLabs. The enclosure is currently being designed.
- [redacted] quote for handheld wand optical components @[redacted]. This item estimates the cost of the lenses, optical fibers, light guide, mirrors, filters, sensors, etc. necessary to construct the wand portion of our device. These are not sole source vendors. We have all certifications and agreements necessary to begin work with these components immediately. This was not a competitive bid; we chose these distributors and OEMs due to our familiarity with their products from our previous projects.

- [redacted] quotes for machined components in the handheld wand is @[redacted]. These are optomechanics that precisely secure the lenses within the wand and the outside housing. These are not sole source vendors. We have all certifications and agreements necessary to begin work with these components immediately. This was not a competitive bid; we chose [redacted] due to our familiarity with their products from our previous project work.
- Machined fixtures and tools are required for assembly of the wand. [redacted] quotes for machined tools is @[redacted].
- The wand housing will be molded or 3-D printed with subsequent machining. Potential supplier is [redacted]. Estimate @[redacted] is based on prior experience working with supplier [redacted]. The enclosure is currently being designed.
- [redacted] quote for miniature 2mm MEMS scanner @[redacted]. This mirror scans the beam and is Aluminum coated to maximize reflection of the 780nm excitation light. We have all certifications and agreements necessary to begin work with these components immediately. This was not a competitive bid; we chose Mirrorcle due to our familiarity with their products from our previous projects.
- [redacted], quote for the disposable molded silicone cap @[redacted]. This item estimates the cost of the twenty molded caps and associated packaging to be provided with each device. This is not a sole source vendor. We have all certifications and agreements necessary to begin work with these components immediately. This was not a competitive bid; we chose this supplier due to our familiarity with their capabilities from our previous projects.
- [redacted] curing station and radiometers @[redacted]. This equipment utilizes UV light for the fast curing of UV adhesives for manufacturing and assembly processes. This is not a sole source vendor. [redacted] is a well establish supplier of UV cure systems and adhesives; we chose this supplier due to our familiarity with their products from previous experience.
- Material cages for part storage @[redacted] to be acquired from local lab supplies and equipment vendor, likely [redacted] or similar vendor.
- Clinical study recruitment and office materials estimated @[redacted]. Based on our experience with multi-site studies, we believe this amount will be sufficient to produce the necessary recruiting materials and administrative supplies for the proposed clinical study.

F.3 Consultants: ([redacted]).

Name	Description of service	Unit Cost	Units	Total Cost	Year 1	Year 2	Year 3
[redacted]	Electrical Engineering / Hardware design	[redacted]	15	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	GUI Software Development and testing	[redacted]	12	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	Regulatory and Clinical Affairs	[redacted]	18	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	Manufacturing/Supply Chain	[redacted]	18	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	Quality Engineering, Quality Systems	[redacted]	20	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	Quality Systems support, Document Control	[redacted]	16	[redacted]	[redacted]	[redacted]	[redacted]
			Category total cost	[redacted]	[redacted]	[redacted]	[redacted]

We have budgeted [redacted] for consultant services. Our anticipated consultant costs are for hardware and software design completion and testing, regulatory and clinical support, and manufacturing and quality systems readiness and documentation. (See attached Letters of Support).

Additionally, we require input from our consulting dermatologist(s)to guide our clinical assessments and lend interpretation on anatomical features in our images in support of Aim 2 (See Letters of Support). However, we will pay for the services of our physician consultants with outside funding, so their costs are not included in this proposed budget.

Our consultants are contracted on an hourly basis up to 16 hours/week, and are available for in-person, phone, and email conversations and feedback. Any additional hours beyond those budgeted here will be paid for with outside funding to support the proposed Aims.

Consultants

[redacted] **– 600 hrs @ [redacted]/hr).** [redacted] serves as [redacted] – a medical device hardware and electrical engineering design firm. [redacted], [redacted] provides embedded engineering consulting services to medical device companies at all stages of product development. [redacted] has over 30 years of design and development experience with a variety of medical and non-medical electronic systems.

[redacted] **– 480 hrs @ [redacted]/hr).** [redacted] is the founder of [redacted] – a medical device software development company. [redacted], [redacted] provides graphical user interface (GUI) design and development services to medical device companies from initial product development through commercialization. [redacted] has worked as an independent consultant and as a full-time employee for several medical device companies and has extensive experience with a broad set of programming languages, tools and platforms in his nearly 20 years as a software designer and developer.

[redacted] **– 180 hrs @ [redacted]/hr).** [redacted] serves as Principal of [redacted] – a Regulatory and Clinical Affairs specialty group. [redacted] provides clinical and regulatory consulting services to medical device companies at all stages of product development. [redacted] has experience with PMA and 510(k) products across a broad spectrum of indications. [redacted] has over a decade of experience in the medical device space. [redacted] has served as Senior Vice President of Clinical Affairs at [redacted], Vice President of Regulatory and Quality affairs at [redacted], and as the Regulatory Affairs manager at [redacted] holds a BS in Biochemistry from UC Davis, and a JD from John F. Kennedy University.

[redacted] **– 720 hours at [redacted]/hr).** [redacted] has broad operations experience including manufacturing engineering, project management, and supply chain management. She is a demonstrated leader of teams of technical professionals and manufacturing resources. [redacted] is a proven driver of project implementation from initiation to delivery. She has participated in multiple new product launches, process validations, manufacturing transfers, a has been responsible for overseeing contract manufacturers and well as internal manufacturing teams.

[redacted] **– 800 hours at [redacted]/hr).** [redacted] is a hands-on consultant in the areas of Quality and Operations. He provides quality assurance and quality engineering support for both start-up and commercialization-stage companies. [redacted] has deep experience in product testing, such as sterilization validation, biocompatibility, design validation, human factors validation, and transit/shelf life testing. He has also completed numerous writeups to support 510(k) submissions and has led multiple Quality Systems implementations. He has expertise in the medical device risk management process, including the development and maintenance of risk management files. [redacted] has conducted many internal audits and supplier audits, has experience with electrical safety testing and certification process (IEC 60601-1), and has completed numerous due diligence reviews for Quality Systems, Quality Engineering, Manufacturing and Supply Chain.

[redacted] **– 640 hours at [redacted]/hr).** [redacted] is an independent Quality system and Document Control consultant with several years of experience supporting product development teams in the medical device industry. She has worked closely with R&D and Manufacturing team members to implement and maintain the necessary procedures and policies to meet quality and regulatory compliance during the development and testing of clinical devices. She also has experience in preparing and managing clinical trial documentation. She will be a valuable resource during both the manufacturing of the clinical devices in Aim 1 and during the execution of the clinical trial in Aim 2 of our proposed project.

[redacted] ([redacted] – 0 hours @ [redacted]/hr). [redacted]ron, who recently joined [redacted] as its Medical Director, is a dermatologic surgeon and former director of the UCSF High Risk Skin Cancer Program, which cares for patients who are at risk for skin cancer due to organ transplant, leukemia, lymphoma or genetic syndromes. She specializes in an advanced procedure called Mohs micrographic surgery to treat certain types of skin cancer. [redacted] also is an expert in facial reconstruction following surgery, laser surgery, dermatology for organ transplant recipients and for those with genetic risk factors for skin cancer. In addition, she performs cosmetic procedures including Botox and soft tissue fillers.

[redacted]'s research interests include studying skin cancer, particularly in patients who are immunosuppressed. She earned a medical degree at Weill Medical College of Cornell University and a doctorate at The Rockefeller University. She completed a residency in dermatology and a fellowship in Mohs micrographic surgery and procedural dermatology at UCSF. [redacted] is the current Vice President of the International immunosuppression & Transplant Skin Cancer Collaborative (ITSCC), an organization of dermatologic surgeons, clinicians and researchers from multiple disciplines dedicated to advancing the care and understanding of skin cancer in organ transplant recipients.

[redacted] ([redacted] – 0 hours @ [redacted]/hr). [redacted] joined Stanford Medicine in 2012 and serves as Director and Chief of Medical Dermatology for Stanford Health Care (SHC) while also spearheading the dermatology department's efforts around network development, digital health, quality/safety/performance improvement, and value-based care. He is active in a number of leadership roles within the organization including co-chairing the Clinic Advisory Council, a forum of medical and executive leaders of Stanford Health Care's Ambulatory clinics, and as a Service Medical Director.

His passion for melanoma, early cancer detection, and improving care delivery drives his efforts and research around leveraging advances in machine learning and artificial intelligence to increase the breadth of populations that can be reached. He conducts research on and engages in collaborations around interventions that layer advances in machine learning on digital health capabilities to enhance access, quality, and value of dermatologic care. He chairs the American Academy of Dermatology's Task Force Committee on Augmented Intelligence.

[redacted] graduated magna cum laude from Harvard University and worked in investment banking; mergers and acquisitions at JP Morgan before going on to earn a combined medical and business degree at Tufts University. During medical school, he was a member of the Alpha Omega Alpha honor society. [redacted] then performed his residency at the Harvard Dermatology Residency Training Program where he served as chief resident.

[redacted] – 0 hours @ [redacted]/hr). [redacted] received his bachelor and medical degrees from Harvard University. He completed his medical internship at Columbia University Medical Center and his dermatology residency at University Hospitals-Case Western Reserve School of Medicine before pursuing a dermatopathology fellowship at the University of Pennsylvania. Board certified in dermatology and dermatopathology, [redacted] practices clinical dermatology and interprets slides as a dermatopathologist. His research interests include the medical applications of artificial intelligence, cutaneous lymphoma, and the cutaneous side effects of targeted therapies. [redacted] served as co-PI on a research project featured on the cover of Nature Magazine. This work has appeared in the Wall Street Journal, Bloomberg Magazine, and PBS NOVA.

F.6 Equipment or Facility Rental/User Fees: ([redacted] – 36 months @ [redacted]/month).

Rent	Description of service	Unit Cost	Units	Total Cost	Year 1	Year 2	Year 3
Fogarty Institute – Sobrato Pavilion	FII incubator shared facility	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
		Category total cost		[redacted]	[redacted]	[redacted]	[redacted]

We pay a monthly fee of [redacted] for access to the Fogarty Institute for Innovation. We are requesting [redacted] per month for the proposed project period to support facility rental costs.

F.8 Professional R&D / Low-volume manufacturing services: ([redacted]).

Professional Manufacturing Services: Minnetronix or Tensentric	Unit cost	Units	Total	Year 1	Year 2	Year 3
Design review and iteration of existing EnSpectra system	[redacted]	1	[redacted]	[redacted]	[redacted]	[redacted]
Build 8 clinical trial ready systems (material handling, assembly labor and fixtures)	[redacted]	1	[redacted]	[redacted]	[redacted]	[redacted]
Conduct Clinical V&V Test and Release of systems	[redacted]	1	[redacted]	[redacted]	[redacted]	[redacted]
Distribute systems to clinical sites	[redacted]	1	[redacted]	[redacted]	[redacted]	[redacted]
Provide technical support as needed during clinical trial	[redacted]	1	[redacted]	[redacted]	[redacted]	[redacted]
	Category total cost		[redacted]	[redacted]	[redacted]	[redacted]

We will work with a medical device manufacturing partner to build, test and release the clinical devices that will be designed to meet the needs of our first-generation commercial product. Our selected partner will have optical, mechanical, and electronics expertise and a track record of supporting clients to move through product development and device manufacturing and testing for clinical trials and 510(k) submissions. With the assistance of the selected partner, we will confirm and finalize the design of the system developed in our successful Phase II proposal to improve component reliability, simplify assembly, and increase the performance of our technology. Our partner will participate in the manufacturing and testing of 8 units constructed under a quality system and will assist with producing the necessary verification and validation testing documentation to support the proposed reimbursement trial(s) as well as a likely 510(k) submission(s).

Budgetary quotes for services and letters of support from two potential partners ([redacted]) have been attached to the proposal.

F.9 Clinical trial expenses: ([redacted]).

Clinical trial expenses	Description of service	Unit Cost	Units	Total Cost	Year 1	Year 2	Year 3
Institutional Review Board (IRB)	Initial reviews, renewals, other reviews at 6 clinical sites	[redacted]	6	[redacted]	[redacted]	[redacted]	[redacted]
Contract Research Org (CRO)	Site qualification, contracting, onsite visits, monitoring, and project mgmt	[redacted]	6	[redacted]	[redacted]	[redacted]	[redacted]
Electronic data capture	Encrypted data collection in accordance with HIPAA	[redacted]	1	[redacted]	[redacted]	[redacted]	$15,000
Clinical site payments	For physician investigator and site coordinator activities	[redacted]	6	[redacted]	[redacted]	[redacted]	[redacted]
			Category total cost	[redacted]	[redacted]	[redacted]	[redacted]

We anticipate expenses with the execution of an approximately 300 patient clinical trial. We will obtain IRB approval at each of six sites, which we estimate at [redacted] per site for the initial review and follow on interactions. We also anticipate either hiring a clinical associate or paying for a part time CRO to ensure adherence to our clinical protocol and oversee patient recruitment at a cost of [redacted] per site. We will utilize encrypted, electronic data capture in compliance with HIPAA regulations which we estimate will cost [redacted] for the six sites. Finally, we will compensate the investigators for their involvement with patient recruitment and delivery of clinical data such as pathology reports, patient demographics etc. We estimate a total cost of [redacted] per site for investigator, site coordinator, and patient reimbursement.

G Direct Costs.

Our direct costs total [redacted] for key personnel salaries, equipment, travel, materials and supplies, consultant services, rent, professional R&D/Mfg services, and clinical trial expenses.

		Total	Year 1	Year 2	Year 3
Key Personnel		[redacted]	[redacted]	[redacted]	[redacted]
Equipment		[redacted]	[redacted]	[redacted]	[redacted]
Travel		[redacted]	[redacted]	[redacted]	[redacted]
Materials/Supplies		[redacted]	[redacted]	[redacted]	[redacted]
Consultants		[redacted]	[redacted]	[redacted]	[redacted]
Rent/Facilities		[redacted]	[redacted]	[redacted]	[redacted]
Contract R&D/Mfg		[redacted]	[redacted]	[redacted]	[redacted]
Clinical Trial		[redacted]	[redacted]	[redacted]	[redacted]
Total Direct Costs		[redacted]	[redacted]	[redacted]	[redacted]

H Indirect Costs

We are not requesting any indirect costs for fringe benefits on salaries of key personnel in this proposal.

I Direct and Indirect Costs ([redacted]).

J Fee. NA

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		0.00
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
Section C, Equipment		[redacted]
Section D, Travel		[redacted]
1. Domestic	[redacted]	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		[redacted]
1. Materials and Supplies	[redacted]	
2. Publication Costs	0.00	
3. Consultant Services	[redacted]	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	[redacted]	
7. Alterations and Renovations	0.00	
8. Other 1	[redacted]	
9. Other 2	[redacted]	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		[redacted]
Section H, Indirect Costs		0.00
Section I, Total Direct and Indirect Costs (G + H)		[redacted]
Section J, Fee		0.00
Section K, Total Costs and Fee (I + J)		[redacted]

Funding Opportunity Number: RFA-CA-20-033 . Received Date: 2020-08-30T20:48:47.000-04:00

OMB Number: 4040-0010
Expiration date: 12/31/2022

SBIR/STTR Information

Agency to which you are applying (select only one)*

❍ DOE ● HHS ❍ USDA ❍ Other:

SBC Control ID:* 000418136

Program Type (select only one)*

● SBIR ❍ STTR

❍ Both (See agency-specific instructions to determine whether a particular agency allows a single submission for both SBIR and STTR)

Application Type (select only one)*

❍ Phase I ❍ Phase II ❍ Fast-Track ❍ Direct Phase II ❍ Phase IIA ● Phase IIB ❍ Phase IIC

❍ Commercialization Readiness Program (See agency-specific instructions to determine application type participation.)

Phase I Letter of Intent Number:

* Agency Topic/Subtopic:

Questions 1-7 must be completed by all SBIR and STTR Applicants:

1a. Do you certify that at the time of award your organization will meet the eligibility criteria for a small business as defined in the funding opportunity announcement?* ● Yes ❍ No

1b. Anticipated Number of personnel to be employed at your organization at the time of award.* 5

1c. Is your small business majority owned by venture capital operating companies, hedge funds, or private equity firms?* ❍ Yes ● No

1d. Is your small business a Faculty or Student-Owned entity?* ❍ Yes ● No

2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies?* ❍ Yes ● No

If yes, insert the names of the Federal laboratories/agencies:*

3. Are you located in a HUBZone? To find out if your business is in a HUBZone, use the mapping utility provided by the Small Business Administration at its web site: http://www.sba.gov * ❍ Yes ● No

4. Will all research and development on the project be performed in its entirety in the United States?* ● Yes ❍ No

If no, provide an explanation in an attached file. Explanation:*

5. Has the applicant and/or Program Director/Principal Investigator submitted proposals for essentially equivalent work under other Federal program solicitations or received other Federal awards for essentially equivalent work?* ❍ Yes ● No

If yes, insert the names of the other Federal agencies:*

6. Disclosure Permission Statement: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and email address of the official signing for the applicant organization to state-level economic development organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?* ● Yes ❍ No

7.Commercialization Plan: The following applications require a Commercialization Plan: Phase I (DOE only), Phase II (all agencies), Phase I/II Fast-Track (all agencies). Include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions.*

Attach File:* COMMERCIALIZATION_PLAN.pdf

OMB Number: 4040-0001
Expiration date: 10/31/2019

SBIR/STTR Information

SBIR-Specific Questions:

Questions 8 and 9 apply only to SBIR applications. If you are submitting ONLY an STTR application, leave questions 8 and 9 blank and proceed to question 10.

8. Have you received SBIR Phase II awards from the Federal Government? If yes, provide a company commercialization history in accordance with agency-specific instructions using this attachment.* ● Yes ❍ No

Attach File:* Commercialization_letter_previous_grants.pdf

9. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?* ● Yes ❍ No

STTR-Specific Questions:

Questions 10 - 12 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 10 - 12 blank.

10. Please indicate whether the answer to BOTH of the following questions is TRUE:* ❍ Yes ❍ No

(1) Does the Project Director/Principal Investigator have a formal appointment or commitment either with the small business directly (as an employee or a contractor) OR as an employee of the Research Institution, which in turn has made a commitment to the small business through the STTR application process; AND

(2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project?

11. In the joint research and development proposed in this project, does the small business perform at least 40% of the work and the research institution named in the application perform at least 30% of the work?* ❍ Yes ❍ No

12. Provide DUNS Number of non-profit research partner for STTR.*

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COMMERCIALIZATION PLAN

Statement of Need

EnSpectra's technology represents a paradigm shift in cellular imaging with potentially far reaching implications. Innovations of this nature generally require greater clinical evidence for both regulatory and reimbursement agencies when compared to incremental technologies. Unfortunately, constrained resources can slow clinical research or force companies to gather evidence sequentially for each respective agency, resulting in protracted commercialization timelines that delay patient access. We at EnSpectra are enthusiastically seeking this Phase IIb award to accelerate our commercialization timeline by building evidence for both FDA clearance and reimbursement coverage concurrently.

We believe our "Valley of Death" extends until we reach an Evidential Tipping Point- a large randomized, well-controlled clinical trial demonstrating that our images are equivalent to traditional skin biopsy (non-inferiority). This Biopsy Equivalency Study (BES) will support reimbursement of our technology and expansion of our indications for use. With the help of this Phase IIB award, we can shorten our "Valley of Death" by initiating this critical study ahead of our series B financing (see Fundraising Plan). We have successfully raised a matching series A, but completing the BES requires additional funds for technical innovation and clinical trial activities. Without this Phase IIB award, we will need to postpone the BES until we close our series B financing. We will also have to fold the BES costs into the series B round, possibly making it harder to raise. This fundraising driven start-and-stop dynamic causes substantial inefficiencies that will delay commercialization.

This Phase IIB award will increase our appeal to series B investors and accelerate our fundraising timeline. Future investors and potential corporate partners will benefit from the shortened "Valley of Death" and reduced financial burden to achieve commercialization. It is unclear how detrimental the Covid-19 pandemic will be to healthcare innovation, but it is likely to make fundraising even more difficult for innovative imaging technologies. This award would validate our company as a leader in the field, add to our current fundraising momentum, and make us more likely to attract additional funding to ultimately succeed.

Fundraising Plan

At EnSpectra, we have demonstrated a successful pattern of fundraising for our unique opportunity. In an era where financing innovative technologies, and particularly imaging technologies, is becoming increasingly difficult, our fundraising record is a strong validation of our commercial opportunity and places us in a small class of venture backed startups. Most recently in August 2019, we closed a matching Series A equity financing of [redacted] million via the sale of convertible preferred stock that serves as an almost 2:1 match for this Ph IIB (see support letters). Our investors include institutional venture capital funds, a large family office, a potential future corporate partner [redacted] and several high-net worth individuals. We believe the experience and contacts gained through our successful fundraising to date have prepared us for successful future capital raises.

We anticipate raising two more rounds of financing (totaling [redacted] million in equity capital) to achieve full commercialization (Table C3). These financings will include a Series B of [redacted] raised in [redacted] driven by the completion of our trial ready clinical system, and; a Series C of [redacted] to [redacted] in [redacted], driven by the milestones of our first FDA clearance and positive clinical results from the Biopsy Equivalency Study (Aim 2 of this grant application).

Within the Series B and C financings, we expect our current investors to participate and help us recruit new investors. Our goal will be to seek new investors (later stage institutional venture capital funds) to lead these rounds, specifically venture funds that can invest large sums with proven records of guiding commercial stage companies.

Use of third-party investment funds

Third party funds raised during the project period will be used to support key efforts and programs required for our commercial success. Pilot clinical studies will be paid for by these third-party funds, in addition to expense of developing both clinical and commercial user interfaces, followed by an extensive development program relating to advancing machine learning (ML) algorithms with our technology. Additionally, we will rely on third-party investment to fund the building of early clinical systems for our initial pivotal clinical study to support our first 510K filing (see Regulatory). Finally, third-party funding will be used for the file preparation

and filing of this initial 510K, which will provide initial access to the US market. Our series A match carried no tranches, restrictions, triggers, or milestones to future payments.

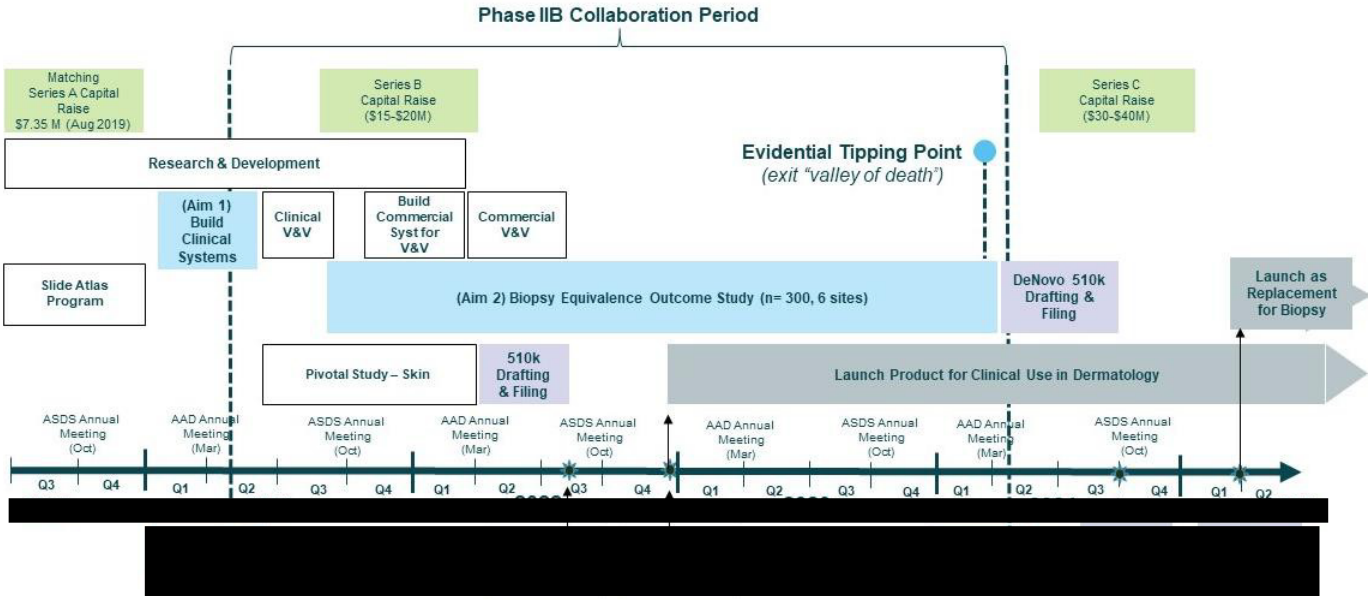


Figure C1. Key commercialization, fundraising, product development, and clinical activities timeline

SBIR Commercialization History

We originally incorporated in Delaware under the name of Zebra Medical Technologies and have leveraged early revenue opportunities to decrease financing risk and burden from the beginning. Early in our development, we generated over [redacted] in revenue in sales through our related muscle imaging technology for scientific research. We used this capital to conduct our first investigations into in vivo skin imaging and subsequently chose to forgo muscle imaging in favor of our skin cancer opportunity. We have received prior Phase I and Phase II SBIR grants from the NCI of the NIH in support of our skin imaging technology. We have not yet generated revenue from our skin imaging technology or any SBIR funded activities.

NCI Phase I: [redacted]

NCI Phase II: [redacted]

SBIR related revenue: [redacted]

Value of the SBIR Project, Expected Outcomes, and Impact

Our noninvasive, read-ready, digital platform will represent a major innovation over the destructive, invasive, and protracted cellular imaging methods used to diagnose solid tumors for the past 150 years. Direct digitization of cellular images from live tissue will enable substantial medical benefits such as (1) accessing histology for earlier diagnosis, surgical planning, and longitudinal monitoring, (2) limiting errors during tissue processing, and (3) sharing image data across clinician teams with the potential for augmented analysis.

EnSpectra Health (formerly Zebra Medical Technologies) Innovations in Phase I, II, and III

Our portable MPM microscope is a single integrated platform designed to work in the hands of practicing clinicians that combines multiple innovations, such as miniaturized fiber delivery of ultrafast pulsed laser excitation, multi-spectral signal generation and processing, MEMS actuated laser scanning, and rapid cross-sectional imaging with subcellular resolution. Each of these innovations is a substantial scientific achievement in its own right, and the combined system is truly one of a kind. We believe this unique combination of features is necessary for mainstream adoption in dermatology.

This Phase IIB proposal is a continuation of funded Phase I and Phase II projects. In Phase I, we proved the feasibility of our unique approach by constructing a portable MPM skin imaging microscope and testing the device in patients with basal cell carcinoma (BCC). We demonstrated successful sub-micron imaging of basal cell tumors in vivo with a handheld microscope that displayed real time, cross-sectional color views of the patient's skin while counteracting motion artifacts. In Phase II, we expanded this success through the continued development of our MPM system, adding custom electronics, solid state optical sensors, and an integrated FPGA architecture to boost data volume for ML applications. We further developed a fully

automated ex vivo slide scanning embodiment to create a library of paired MPM (unstained) and H&E stained slide images to benchmark our imaging characteristics against traditional histology. Not only does our scanner give us the ability to build standardized data sets for clinical training, it also allows us to test imaging feasibility in other tissues of interest and sets the stage for future intraoperative and bedside applications (Figs. R7 and C2).

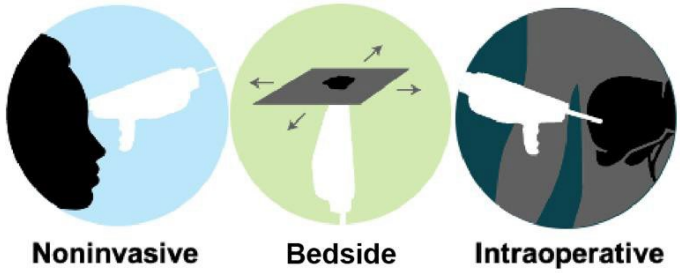


Figure C2. Platform vision for clinical MPM digital pathology

Within the Phase IIB funding period we will further innovate our device design and user interface, build a suite of clinical systems, and execute a Biopsy Equivalency Study. The results of this clinical study will be critical for securing a diagnostic claim from the FDA and establishing payer and Medicare reimbursement for the diagnostic use of our System (Fig. C1). In Phase III, we will introduce a limited number of MPM systems to test the market while conducting more advanced post-market clinical trials to expand our approved device use to include diagnosis of NMSC and other conditions.

Unmet need: Early detection of NMSC

We will initially address the diagnostic errors and associated costs of detection and treatment of nonmelanoma skin cancer (NMSC). NMSC, which includes both basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), accounts for over 75% of cancer in the United States and its incidence continues to rise[1,3]. Both BCC and SCC are destructive and require removal to spare healthy skin and underlying structures. In contrast to melanoma, NMSC rarely metastasizes and any one lesion poses a lower mortality risk to patients than a melanoma lesion. However, because NMSC has a far higher incidence[1], it accounts for the majority of skin cancer deaths, with SCC alone causing more than twice as many fatalities as melanoma each year[30,31]. SCC is particularly concerning in patients with high frequency skin cancer (HFSC), such as organ transplant patients, who are 100 times more likely than the general population to develop SCC[32]. Economically, NMSC also accounts for the majority of skin cancer biopsies, surgical treatments, and costs[33]. Part of this cost stems from how suspected NMSC is monitored, diagnosed, and treated.

Because NMSC is rarely fatal, sometimes concerning lesions are monitored and re-inspected at a future office visit. Consensus guidelines acknowledge that the need for biopsy is counterbalanced by a preference to minimize discomfort, trauma, risk of infection or dehiscence, scar, or loss of function caused by the biopsy[34,35].Unfortunately, if these lesions are ultimately found to be malignant, they are larger[5] at the time of treatment, which increases the invasiveness, complexity, fatality risk, and cost of treatment at the time of removal. Treatment costs for larger NMSCs increase due to more complex closures and follow-up revision surgeries, the inability to use lower cost treatment alternatives like electrodessication and curettage or wide excision[36], and the use of additional surgical stages if Mohs surgery is used. We need a better way to detect NMSC earlier than with clinical inspection and surgical biopsy alone.

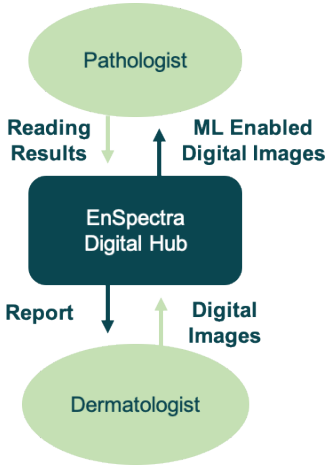


Figure C3. EnSpectra flow of care with noninvasive pathology. With enough evidence, eventually biopsy confirmation may not be needed to proceed directly to treatment.

EnSpectra Health is developing technology for dermatologists to use in the office to provide immediate, point-of-care histopathological information about suspected NMSC (Fig. C3). Interviews with dermatologists across the country revealed that our technology aligns with their needs (Table C1). By eliminating the invasiveness of a biopsy, clinicians will be able to inspect more suspicious lesions at earlier and more frequent intervals and identify NMSC earlier (Fig. C3). Within NMSC, there are three specific use-cases we believe represent the greatest need for our technology. The first, HFSC, affects those with compromised immune systems and leads to much higher incidence and even long term fatality risk from NMSC[37]. There are over 600,000 patients with HFSC that regularly see their dermatologist to assess which of their multiple presenting lesions are benign or malignant[38]. With multiple lesions assessed at each office visit, these patients often carry high biopsy burdens and a need to identify cancer as early as possible. A second meaningful use-case is for pre-surgical planning to improve clinical margin assessment on standard excisions. We estimate that

up to 2.0 million excisional procedures could benefit from the use of non-invasive, read-ready imaging for surgical planning each year. Our solution could allow dermatologists to effectively remove smaller lesions in areas of high risk for recurrence, reducing the "watch and wait" period and the need for more specialized and expensive services, such as a Mohs surgery. The third specific use-case is earlier detection of small lesions within the mask region of the face which experiences the highest frequency of lesions[39]. Clinicians often employ a "watch and wait" strategy in this region, but our technology would allow for earlier diagnosis and treatment of NMSC as there is literally no harm in looking.

Early NMSC detection aligns with the values of all key stakeholders: (1) patients, (2) physicians, and (3) payers. Patients value earlier diagnosis, earlier intervention, and avoidance of unnecessary pain and scarring from biopsies. Dermatologists value delivering superior care and improving operational efficiency. Moreover, retaining NMSC patients for simpler treatments such as excision, rather than referring patients out-of-office to a specialist Mohs surgeon, represents a lucrative revenue stream to dermatologists. Finally, payers value earlier and therefore lower cost treatment of smaller cancers. These benefits of our solution make the likelihood of clinical adoption and commercialization high.

Table C1. Representative interview responses of practicing dermatologists from EnSpectra's national market research.

Dermatologist Location	% cases involving skin cancer	If you could get noninvasive pathology images during the visit, would that be valuable to you?
Concord, MA	A lot. Half at least.	That would be amazing. The doctors would love it. The patients would love it. That sounds like a dermatoscope on steroids. You're not doing unnecessary biopsies, but you can relieve the patient's anxiety. Waiting weeks for a test was OK in the 1950s and 1960s, but now it's 2017. If you can get the results in 15 minutes to an hour, that would be great.
Los Angeles, CA	20% for skin cancer screening.	Without a doubt. Decreased cost, downtime, pain, scarring. There have been a lot of technologies that say they're good but they're not, so I'm very skeptical. Colleagues have let me down. To be convinced, I would need a head-to-head study looking at biopsy-proven melanomas and SCCs, and look at what degree the noninvasive technology agrees with traditional pathology.
Raleigh, NC	30%.	The advantage for me would be to know the diagnosis when you're sitting with the patient so you can prepare them for what they have instead of over the phone. Depending on logistics and economics of this, if it's NMSC, we could improve our acumen at reading pathology. You could still send the slide to the pathologist. It would help ensure I biopsied the right part of the lesion. It could help guide treatment.

To further increase our likelihood of success and impact, we will build early clinical evidence and market penetration by first pursuing FDA clearance for use of the device 'to assist in clinical decision making' (i.e. screening). Our market research revealed that dermatologists and research institutions conducting clinical trials prefer studies with FDA-cleared devices. Furthermore, evidence of adoption of a new technology is typically required before positive payment decisions can be made by insurers and other payers. Finally, the FDA clearance indication we are pursuing will streamline technology access for diverse areas of skin research across the United States.

Beyond earlier diagnosis of NMSC

While our focus is to improve patient care through earlier NMSC diagnosis, our invention has potential clinical value beyond NMSC, beyond skin disease, and even beyond cancer (Fig. C2). Several emerging topical treatments and drugs for the treatment of NMSC and other dermatologic conditions are ideal for pairing with our technology. With our device, histopathology could become a proactive component of therapy for the first time in 150 years, guiding the application of topical drugs and monitoring effectiveness, resulting in completely noninvasive treatments. For intraoperative applications, we are actively developing a micro endoscope objective that is the same size as a liver or breast biopsy needle (not part of this SBIR grant). Finally, we plan to develop our fully automated scanning system for immediate, unstained, bedside histopathology and cytology.

The application of machine learning (ML) techniques to digitized slide images has experienced accelerated traction in recent years. The Covid-19 pandemic has bolstered industry trends indicating a shift in mainstream adoption of digital pathology. However, unlike other companies limited to scanning slides, the flexibility and

noninvasive nature of our microscope will allow us to create datasets that no other company can duplicate. Major industry players (Google, Philips, Zeiss, and Leica) as well as emerging pathology software companies (PaigeAI, PathAI, and Proscia) are strong contenders to partner with EnSpectra in Phase IIB and beyond.

Foreseeing this digital shift, we innovated in our Phase II system to substantially boost our data throughput capacity with a roaming image rate of 9.4 frames/s and a high-resolution streaming rate of 1.2 frames/s. At these speeds, we can generate 1,000 high resolution images every 15 minutes. This boosted data rate will allow us to create trainable-scale datasets (10,000's of images) for use with ML algorithms. We can also generate internal control data (by scanning skin near and around a lesion) for better tissue context and ML performance which is impossible to do with glass slides. Techniques leveraging recurrent neural networks with weakly supervised training would be ideally suited for the data we produce[40].

Company

EnSpectra Health focuses on improving patient care through need-driven imaging innovation. Nearly every known disease in the body originates at the cellular scale before becoming clinically apparent, yet we still lack the ability to easily view cellular tissue. This widespread clinical need motivates our team and technology innovation. EnSpectra Health is a spinout from Stanford University, where CEO [redacted] invented the first version of portable multiphoton microscopy during his PhD research with Professors [redacted] and [redacted][41–44]. After serving as a lecturer in the Bioengineering department at Stanford, [redacted] co-founded Enspectra Health and dedicated his efforts to the opportunity of in vivo cellular imaging. EnSpectra was accepted into the prestigious Fogarty Institute that provides a wealth of facilities, mentorship, networking, and corporate development training to launch early stage innovative companies into commercial viability. EnSpectra licensed three patents from Stanford University around core innovations in MPM imaging in thick biologic tissues, fiber delivery of ultrashort laser pulses, and remote focusing through thick tissue.

In [redacted], we successfully raised a Series A round of [redacted] million to accomplish five primary objectives: grow our team; refine our prototype into a clinical product; build several clinical ready systems; develop a library of slide images of targeted skin diseases comparing EnSpectra MPM to traditional histology, and; conduct an accuracy assessment on our library images amongst blinded readers. We have previously received a Phase I ([redacted]) and Phase II ([redacted]) award for this project through the NIH. The funds from this grant will directly support the further development of our system and the execution of a large Biopsy Equivalency Study.

Our core competencies in project leadership, optical and mechatronics design, clinical research, commercialization, communication, and operations have enabled our rapid progress that appeals to investors and has allowed us to sustain a well-financed operation. Beyond hardware innovation, we foresee an emerging digital health opportunity for EnSpectra and we plan to grow our expertise in the field of ML algorithms. Our current full-time team includes [redacted] Our academic co-founders, [redacted] provide invaluable technical guidance and entrepreneurial experience. We complement our core team skills through consultants that focus in key areas such as regulatory, clinical, statistics, quality control, and reimbursement affairs (see Letters of Support). We will continue to grow our company prudently, building our internal expertise and reducing our dependence on consulting services. As members of the Fogarty and Rosenman Institutes, and collaborators with Stanford Biodesign, we are part of an extensive network that links us to leading industry professionals, future team hires, and investors.

Market, Customer, and Competition

NMSC is an excellent target market for the EnSpectra System due to market size, disease risk, and alignment with current practice workflow

Our product will enable earlier detection of nonmelanoma skin cancer (NMSC), the most common form of human malignancy. Despite increased awareness of the dangers of ultraviolet light exposure, the incidence of skin cancer continues to grow; the annual number of NMSC lesions treated in the US increased every year between 2002 and 2012[1,45]. Projecting this trend to the present, we estimate that 6.5M NMSCs will be treated in the US in 2020.[1]

The annual cost of diagnosis and treatment of NMSC in the US is *$6.5B*[1,33]. Assuming a global market of approximately double this size, NMSC presents a large and growing market opportunity. We chose to initially pursue NMSC as opposed to melanoma for important strategic and commercial reasons:

- *Larger market size*. NMSC represents 98% of all skin cancers. With so many NMSCs and associated procedures, this market aligns with a business model based on recurring revenue and high volume of use.
- *Lower mortality risk*. NMSC is rarely fatal, so an early diagnostic can deliver tremendous value at no

additional risk to the patient. Nearly all the competitors before us have attempted to commercialize technologies for melanoma screening and have struggled to gain commercial traction. In our assessment, the risk of fatality with melanoma is so high that clinicians would require near perfect diagnostic evidence to change their behavior of diagnosing 20 benign lesions for every identified melanoma[46]. NMSC in contrast is biopsied at a rate closer to 2 to 1[1] and it is not uncommon to delay a biopsy of a potential NMSC until a later office visit. Since our device is completely noninvasive, there is no downside to inspecting even moderately suspicious lesions. If we identify a cancer earlier, then the patient, physician, and payers benefit. If the physician believes the lesion is benign, then the physician can inspect it again at the next visit without exposing the patient to additional risk.

- *Alignment with biopsy type*. There are two major varieties of skin biopsies: a shave biopsy and a punch biopsy. Our market research and clinician interviews revealed that NMSC is diagnosed almost exclusively using shave biopsies. This aligns with our imaging depth capabilities as a typical shave biopsy extends only through the epidermis and into the reticular dermis, a total depth of a few hundred microns. In contrast, suspected melanoma is sometimes assessed with punch biopsies to stage depth of invasion.

Within NMSC, there are three specific use-cases we believe represent the greatest need for our technology. We will focus tactically on penetrating these market opportunities first.

High frequency skin cancer (HFSC). HFSC is an affliction of those with compromised immune systems, such as organ transplant patients who can experience 100 times the normal risk of developing skin cancer and higher fatality risk[32,37]. By our estimates, there are over 600,000 patients with HFSC that regularly see their dermatologist to assess which of their multiple presenting lesions are benign or malignant. With multiple lesions assessed at each office visit, these patients often carry high biopsy burdens, and a need for detection as early as possible[38]. We believe our non-invasive, real-time, read-ready system will help dermatologists inspect and monitor more suspicious lesions, finding NMSCs earlier and improving outcomes for the patient.

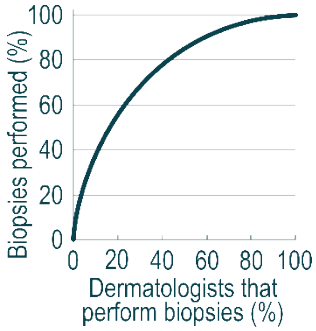


Figure C4. Number of biopsy services billed to CMS by dermatologists (2015 CMS Provider Utilization and Payment database).

Pre-surgical planning to improve clinical margin assessment on standard excisions. We estimate that up to 2.0 million excisional procedures could benefit from the use of non-invasive, read-ready imaging for surgical planning. Our technology would allow general dermatologists to effectively remove smaller lesions in areas of high risk for recurrence (such as the head and neck), reducing the "watch and wait" period and the need for more specialized and expensive services, such as a Mohs procedures.

Earlier detection of small lesions found within the mask region of the face. This mask region of the face experiences the highest frequency of lesions[39] and is an area where clinicians regularly employ a "watch and wait" strategy. Our technology will allow earlier diagnosis and treatment of NMSC within this high-risk area of the face.

Dermatologists, our initial and primary users

Dermatologists are ideally suited for the first introduction of our technology because of their training and experience interpreting NMSC dermatopathology. Dermatologists also spend a large percentage of their time evaluating skin cancer (Table C1). Among dermatologists, NMSC services are concentrated; 50% of biopsies are performed by only 16% of biopsy-performing dermatologists (Fig. C4). Thus, millions of NMSC patient interactions are concentrated into just a few thousand, high-volume offices. A business model based on pay-per-procedure recurring revenue and a low rate system lease has the greatest chance of success versus sales of capital equipment (see Revenue Stream)

Mohs surgeons, dermatologists who specialize in Mohs surgery, are also skilled at interpreting dermatopathology, however they are downstream in the clinical pathway, do not control patient flow, and see fewer patients. Furthermore, Mohs surgeons have less incentive to noninvasively evaluate NMSC and margins because Mohs surgery is highly reimbursed proportional to the size of the lesion.

The majority of NMSC patients are treated by dermatologists in office settings (Fig. C5). In contrast to facility dermatologists, typically in academic settings, private practice dermatologists have greater decision-making power to adopt new technologies since there is no hospital Value Committee. Importantly, these private practice dermatologists also hold more responsibility for the financial solvency of their medical practices; they are more aware of reimbursement policies and adept at evaluating cost-value considerations.

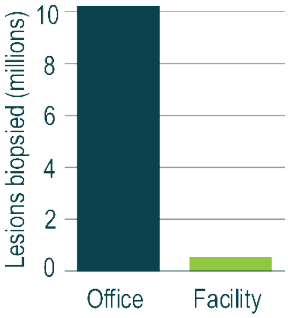


Figure C5. Lesions biopsied, most of which are suspected NMSC, in office versus facility settings (2014 CMS Provider Utilization and Payment database).

Dermatology practices that conduct a mixture of medical and cosmetic services are the most common. They tend to have greater comfort with adopting new technologies and have patients who are typically more motivated to avoid biopsies. We conducted a series of interviews of 25 dermatologists across the United States (Table C1). The majority of the dermatologists we interviewed worked in this style of clinic although the medical-cosmetic proportion in each clinic varied. Dermatologists revealed that although cosmetic procedures tend to be more lucrative per unit time, their fraction of cosmetic procedures fluctuates with the overall state of the economy since cosmetic procedures are dependent on cash payments from patients. In contrast to cosmetics patients, the skin cancer patient population is economy-independent and continues to grow. This is particularly salient during this Covid-19 pandemic when cosmetic and elective services have declined precipitously. Dermatologists rely on skin cancer patients to maintain viable practices because they generate reimbursed procedures. This is a great feature of our strategy to target NMSC because dermatologists can use our product on a patient population that they already serve.

We will initially target sales to dermatologists that do not have anyone in their practice who performs Mohs surgery, as these dermatologists have the most to gain from noninvasive imaging. These non-Mohs dermatology practices represent 73% of practices; they perform 54% of NMSC biopsies (2015 CMS Provider Utilization and Payment database).

Presently dermatologists who do not perform Mohs surgery refer 37% of their confirmed skin cancer patients to external Mohs clinics for surgical treatment (2015 CMS Provider Utilization and Payment database). Our hypothesis is that many of these cancers could be treated without Mohs if they were identified at an earlier stage, when the lesion is smaller (Fig. C3). Because we anticipate our noninvasive technology will be used more frequently than traditional shave biopsies, it is likely malignant lesions will be found earlier when the lesions are smaller. Although NMSC is unlikely to metastasize, the lesions grow substantially over the standard period of time between appointments (6-12 months)[5]. Larger NMSC lesions are also more costly to treat, as lesion and defect size are direct determinants of reimbursement procedure coding.

Mohs surgery is about twice as expensive as wide excision and over 4 times as expensive as lesion destruction performed in dermatology clinics[47]. Shifting more care to the dermatologists, therefore, will save healthcare costs. At the extreme, if we enabled a complete halt to Mohs referrals, it would generate a net savings of [redacted] per year for the healthcare system. The cost savings could be used to reimburse more inspections with our device to increase the chances of earlier detection, so there will likely be a balance between increased inspection with our technology (increasing costs) and a shift towards earlier detection (lowering of costs) that will ultimately determine the reimbursement economics of our technology.

Our technology has a high likelihood of adoption because it can improve operational efficiency and treatment value for dermatologists. Dermatology clinics are typically overbooked and attempt to see as many patients as possible within the confines of operating hours. **If cancer is found earlier, there may be increased opportunities for less complex treatments like wide excision, electrodessication and curettage, and topical pharmaceuticals.**

A common request from time constrained dermatologists is that we design our device to minimize use-time. We observed many biopsy procedures and found dermatologists spend between 5-10 minutes performing a biopsy, with other preparation and procedure time performed by medical assistants. Our device is comparable in use-time to a biopsy because images display on the screen in real time, although the device will replace more procedures than just a biopsy (i.e., sample processing, slide scanning). Our technology can be easily carried from room-to-room and is always ready for use. The time- and ease-of-use of our technology is essential since our business model is based on volume (See Revenue Stream)

FDA 510(k) strategy

Dermatologists are excited about our approach and we have had great reception of our current system. There are four factors dermatologists will require to broadly adopt this technology:

- *FDA clearance*. The dermatologists we spoke with placed high value on FDA clearance to establish the safety of our technology. During the project period of this Phase IIB SBIR grant proposal, we will conduct a pivotal human clinical trial that will support our first 510(k) submission as a diagnostic adjunct. This trial and the

510(k) submission are outside of the scope of this Phase IIB award and will be supported by independent third-party funding (see Finance Plan section).

- *Evidence of clinical accuracy*. A robust body of clinical evidence underpins our success in securing adoption of our technology. Future regulatory clearances and approvals for bolder marketing claims and reimbursement coding, payment, and coverage will also heavily depend on evidence of clinical accuracy. This Phase IIB SBIR grant proposal will support a Biopsy Equivalency Study within NMSC.
- *Dermatology society support*. We will need the support of dermatologic societies that can appeal to their physician members and to payers for coverage on our behalf. FDA clearance will validate our technology and help us win support within these societies. An abstract summarizing our first in vivo data supported by our Phase I SBIR NCI grant was presented at the 2018 American Society of Dermatologic Surgeons conference, which spread awareness of our technology and generated further advocacy from this influential society.
- *Payment by insurance and other payers*. Insurance payment for new technology will be required to fully access the dermatology market. FDA 510(k) clearance will streamline access to payment through already existing CPT codes. To secure payment and coverage, we will require a body of high-quality clinical trial evidence directly assessing diagnostic accuracy for specific disease indications in a well-defined patient population. The Phase IIB grant funds will support the gathering of this clinical trial evidence within the Biopsy Equivalency Study.

Due to the importance of FDA clearance for clinical adoption, we developed a strategy to accelerate clearance through the 510(k) pathway using a first indication as a diagnostic adjunct. This first indication will create an opportunity for early commercialization and will serve as a springboard into a full diagnostic indication. The 510(k) cleared predicate device we will follow is the VIVASCOPE® by Caliber I.D., which uses reflectance confocal microscopy (RCM) as its imaging mechanism[48]. The intended use of the VIVASCOPE® is "to acquire, store, retrieve, display and transfer in vivo images of tissue, including blood, collagen, and pigment, in exposed unstained epithelium and the supporting stroma for review by physicians to assist in forming a clinical judgment" (510(k) summary K180162). The broad language of the intended use statement will apply to various skin diseases, including NMSC and other malignancies, which will streamline clinical studies and adoption.

Although the optical mechanism, physics, and performance of RCM differs from MPM, for purposes of regulatory clearance the two imaging modalities are substantially equivalent; both modalities use low average power infrared light, have similar safety profiles, are intended to be used in similar ways by similar users, image similarly sized microscopic structures (cells), and are used to visualize exposed unstained epithelium. We have completed two pre-submission inquiries (Q-submissions) with the FDA concerning our planned submission and have received confirmation of acceptance of VIVASCOPE® as our predicate device. Additionally, the FDA has confirmed our clinical strategy and trial designs for this initial 510(k) submission. FDA clearance will externally validate the safety of our device and garner trust from the medical community.

Initial reimbursement strategy following our first regulatory approval (with VIVASCOPE® as predicate)
Established category I CPT codes for RCM (96931 to 96936) and assigned payment exceeding that of surgical biopsy enable a path to payment and early revenue for EnSpectra's technology (Table C2). Our strategy is to generate the necessary clinical evidence to gain FDA clearance and receive payment under these codes using the VIVASCOPE as a predicate. Dermatologists could receive premium payment using our device generating more revenue compared to a traditional biopsy.

Although there are established reimbursement codes for RCM, neither Medicare nor large health plans offer coverage under these codes. VIVASCOPE®'s inability to achieve a positive coverage decision stems from the burdensome training required to interpret monochromatic en-face images - over 4,000 hours[49], challenges related to image acquisition of target lesions, and lack of system portability. Our system's color contrasted, cross sectional images will minimize the training burden for pathologists, and the portable architecture provides easy targeting of lesions. These features will promote clinical adoption and superior evidence to achieve a positive coverage and payment decision for EnSpectra Health under these codes.

This is an attractive opportunity to enable early commercialization and market testing while creating a clinical gateway to the evidence necessary for future diagnostic indications. Our strategy appeals to investors by partitioning the risk in a way that makes commercialization much more feasible. The opportunity for earlier revenue will reduce our capital needs and lower our financing requirements, will give us an opportunity to test product-market fit in a real-world setting, and will increase our chances of securing funding.

Regulatory and reimbursement strategy: 510(k) clearance and beyond

Because our initial 510(k) clearance alone is insufficient to encourage widespread clinical adoption, subsequent steps will be taken to develop evidence, introduce the device to the market, and secure reimbursement.

Table C2. Comparison of Category 1 CPT codes for standard of care diagnosis done by dermatologists (biopsy and tissue exam) versus one of the codes for bundled imaging and interpretation with RCM. We will seek to expand RCM codes to include MPM. Dermatologists will gain revenue performing noninvasive imaging (2020 CMS Physician Fee

Procedure	Standard of care	2020 CMS limiting charge	New RCM codes	2020 CMS limiting charge
Sample skin	Biopsy (11102)	[redacted]	Bundled: RCM cellular, subcellular imaging of skin (96931)	[redacted]
Interpretation	Tissue exam (88305-TC)	[redacted]		
Total		[redacted]		[redacted]

- Step 1. Initial clearance and market testing release. *Evidence*. Show device is safe and effective to image skin microstructure in people. *Regulatory goal*. 510(k) clearance to use device as a diagnostic adjunct. *Reimbursement goal*. Achieve positive coverage and payment decision under VIVASCOPE® CPT codes. These activities are outside of the scope of the NCI SBIR Phase IIB grant award and will be supported by existing and additional funding raised through venture capital.
- Step 2. BCC diagnosis release. *Regulatory evidence*. Show diagnostic accuracy of device to discriminate between equivocal lesions of suspected BCC, a subset of NMSC, which is the purpose of Aim 2a of the Research Strategy presented in this Phase IIB grant application *Regulatory goal. De novo* regulatory clearance to use device in place of biopsy for some types of BCC lesions. *Reimbursement evidence*. Demonstrate smaller lesion size at time of detection with EnSpectra Health's technology, 'real-world' patient outcomes (e.g., lower complexity of treatments, smaller defect size) and economics of using the device, which is the purpose of Aim 2b of the Research Strategy presented in this Phase IIB grant application. *Reimbursement goal*. Secure coverage for use of the device to serve in place of a surgical biopsy for BCC.
- Step 3. SCC diagnosis release. Repeat Step 2 for SCC.
- Step 4. Expanded applications and uses. Continue developing technology to provide diagnostic information for other skin malignancies, including melanoma. Continue to expand indications within the NMSC market to assist with surgical planning, margin estimation, evaluating suitability for noninvasive topical treatments, and longitudinal treatment monitoring.

Competitive analysis

The need to improve skin cancer detection is a well-established and unmet clinical need with a large and growing market. We broadly categorize the competition into three groups: alternate physics approaches, superficial photography, and digital surgical pathology (Fig. C6).

Alternate physics approaches include technologies like RCM (Caliber I.D.'s VIVASCOPE®), raman spectroscopy (Verisante's Aura), and other versions of MPM (Jenlab's MPTflex). Thus far, these technologies have failed to integrate into the clinical workflow. Most require laborious setup and extensive interpretation time, produce data in an unfamiliar format, and are excessively costly. Image based solutions, like the VIVASCOPE® and MPTflex generate en face images parallel to the skin surface. These are difficult for clinicians to interpret because histology is typically viewed from a cross section perspective. MPTflex now has an XZ imaging capability, but at 34 seconds per single scan[13], this seems prohibitively slow to survey the skin and is likely subject to severe motion artifacts. VIVASCOPE® further lacks tissue contrast, so it can be very difficult to distinguish features of cancer and regions of cells versus connective tissue. Most of VIVASCOPE®'s signal arises from melanin and Caliber I.D. has advertised an interest in applications for melanoma. As we outlined in this section, it will be extremely difficult to generate clinical confidence in diagnosing melanoma. The MPTflex is described as a portable MPM imaging system, but at 250 kg, according to the JenLab website, it is the size of a large upright piano and is 25 times the mass of our miniature system.

Superficial photography, which includes dermoscopy and total body photography, has gained momentum in recent years, particularly with the popularity of ML neural nets. These companies have proposed products for various customers ranging from patients, primary care physicians, and dermatologists. These companies claim that an ML algorithm with a large enough training base might be able to distinguish features on the skin's surface that are diagnostic of cancer. We find this unlikely because the features of skin cancer are within the cells beneath the surface. With more development these technologies may become useful for skin cancer screening, but diagnostic decision making will require diagnostic quality images, e.g. histopathology.

Digital pathology of scanned, H&E stained slides is also an emerging field. A notable example is the FDA's *De Novo* clearance of the Philips Intellisite system for primary diagnostic use. This clearance suggests regulators and clinicians anticipate a future with at least digitally augmented pathology. Leica, Zeiss, and Roche are developing their own competitive digital slide readers. As exciting as this new field is, these tools still fail to address the core operational inefficiency of histopathology which is the need for a biopsy. Nonetheless, this is a positive sign of enthusiasm on behalf of regulators. This momentum will help us build awareness and acceptance of our own noninvasive digital imaging and pave the way for the addition of ML tools to augment diagnosis in the future. Furthermore, we expect to be an attractive acquisition target for the strategic acquirers in this field. An acquisition or partnership could streamline our pathway to widespread clinical use.

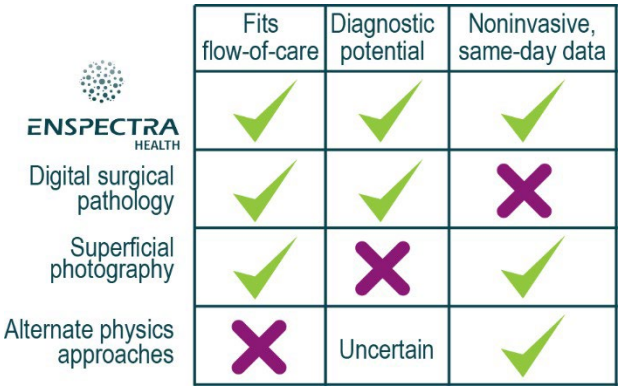


Figure C6. Competitive technologies overview.

Intellectual Property (IP) Protection

A strong and diverse IP portfolio is central to our strategy for success. When we incorporated EnSpectra, we exclusively licensed a suite of [redacted] patents from Stanford University. Within EnSpectra, we have continued to grow our portfolio with new imaging innovations and data processing techniques. We maintain a regular cadence of new submissions and emphasize international Patent Cooperation Treaty (PCT) applications as we anticipate a large global opportunity for our technology. Our specialization in hardware is an advantage because we can link device and apparatus claims to less tangible assets, such as data structures and algorithmic processes. We work closely with [redacted], a world leader in corporate IP to develop a healthy portfolio and assess the competitive landscape in our space. With [redacted], we conducted a freedom to operate (FTO) analysis to methodically search for any IP that would hinder commercialization and our analysis came back clear of any concerning IP. Additional barriers to competitors are our core competencies, fundraising record, and pace of innovation. The unique capabilities within our team will help us stay ahead of our competitors and gain a first-mover advantage in dermatology.

Finance Plan

Maintaining a well-financed operation is one of our central strategies for success. We have achieved substantial milestones that appealed to angel and early stage investors and helped us secure the necessary resources to continue our progress. We have raised over [redacted] to date through a combination of strategic investors, early stage venture capitalists, angel investors and grants. Our financing consists of a [redacted] seed round, a [redacted] Series A convertible preferred stock offering, and a [redacted] Phase I and II NCI SBIR grants.

Table C3. Fundraising plan and use of independent third-party investor funds

Series	Milestone Trigger	Closing Date	Amount ($ million)	Investor Type	Anticipated Use
A	Positive NIH Phase II funding response	[redacted]	[redacted]	Tsingyuan Ventures; Tao Capital Partners; Stanford University; Konica Minolta Inc; F-Prime Ventures; Heuristic Capital; Social Starts.	Support for Phase II project and initial period for Phase IIB, product development
B	Completion of trial ready system	[redacted]	[redacted]	New institutional venture capital with existing investor participation	Pivotal study and 510(k) clearance, ML studies, Biopsy Equivalency Study support
C	FDA clearance and Results from Biopsy Equivalence Study	[redacted]	[redacted]	New late stage institutional venture capital with existing investor participation	Full commercial launch in mainstream dermatology within US market

Production and Marketing Plan

Although an acquisition of EnSpectra by a complementary company may be a positive outcome, we are planning for the full life cycle of our technology. Production and marketing will follow a stepwise process appropriate to our stage of evidence development.

Regulatory and reimbursement strategy: 510(k) clearance and beyond.

- Step 1. Pre-launch market development. *Production*. Advance our technology from prototype to a trial ready product, robust to various users and environments and appropriate for regulatory testing. During this period, we will manufacture systems for our initial 510(k) trial and for regulatory testing purposes. Manufacturing will be partially performed in-house, with EnSpectra assembling the optical wand, while the electronic base station, monitor, and cabling will be completed by a partnering contract manufacturing firm. By working with an experienced partner, we will dramatically decrease regulatory risk and ensure the device we test in our clinical trials meets or exceeds all regulatory standards. These activities are outside the scope of this Phase IIB award and will be supported by third party funding. *Market development*. During this pre-launch phase, we will actively build relationships with Key Opinion Leaders at top teaching institutions across the US. This will include conducting product demonstrations, planning for investigator-initiated studies and seeking collaboration in formulating publication strategies and tactics. Additionally, we will identify and recruit six clinical trial sites for the Biopsy Equivalency Study (Aim 2 of this grant). We have identified approximately 200 clinics within the US that see a disproportionate share of high frequency skin cancer patients. Our priority will be to profile the volume characteristics of these clinics and to build relationships, with the goal of finding at least 20 clinics to trial systems in following initial regulatory clearance.
- Step 2. Initial regulatory clearance and limited market release. *Production*. Advance our technology to a robust commercial product. These activities are outside the scope of this Phase IIB award and will be supported by third party funding. Manufacturing will continue to be partially performed in-house by EnSpectra along with a partnering contract manufacturing firm with substantial medical device experience. *Market introduction*. Following our initial 510(k) clearance, the device will be brought to market in a controlled fashion to test product-market fit, streamline post-market clinical studies, and generate revenue to ease the burden of fundraising. We will initially target between 20 and 30 dermatology group practices and centers-of-excellence that treat a disproportionate share of high frequency skin cancer patients. These clinics will have patient and procedure mixes that will lead to use within our other two target segments of pre-surgical planning for excisions and early detection of small lesions on the head and face. These clinics will have the following characteristics: (1) office-based, (2) perform a mix of clinical and cosmetic dermatology, (3) lack Mohs surgery capabilities, and (4) have patients eager to pay out-of-pocket for noninvasive skin screening. Although we will be using an existing reimbursement code set (Table C2), we anticipate that neither Medicare nor private insurers will cover our systems initially. Consequently, early device use will likely be rebated to promote adoption and patients will pay out-of-pocket. While the purpose of this initial release is to test product-market fit, early sales revenue may relieve some of our fundraising burden. During this step, we will field a small group of three sales representatives to learn how to effectively sell our product in a cost-efficient manner. These learnings will prepare us for a national commercial launch within step 3.
- Step 3. BCC diagnosis release. *Production*. Further advance the technology to limit the cost of goods, enhance ease of use, and enable high-volume manufacturing. Manufacturing at this stage will be transferred to a partnering firm with experience handling high volume production while we develop a body of clinical evidence for diagnostic accuracy of BCC. We do not anticipate that the device design changes needed for high-volume production will necessitate an additional 510(k) submission. This design effort will support a full product launch to support diagnosis of BCC. *Market introduction*. Following regulatory clearance for BCC diagnosis, we will target the top 5% of dermatology clinics – based upon volume - which will dramatically decrease our need for a large sales infrastructure. These 325 clinics account for over 20% of the biopsies performed in the United States (Table C4). This strategy will allow us to gain significant traction and market penetration, without having to indiscriminately market across the country. At this step, we will rapidly grow our commercial organization for a broader, national launch. These activities will include expanding our field sales force to 18 representatives, and four field based Clinical Specialists extensively trained in pathology to assist with training customers. Additionally, we will build a small, market facing Medical Affairs Team that will focus on generating publications, presenting at medical meetings, and supporting investigator-initiated studies at key teaching institutions and dermatology centers-of-excellence.
- Step 4. SCC diagnosis release. *Production*. We expect no redesign will be required to visualize SCC pathology. *Market introduction*. Little change in market introduction will be required. The same clinics that diagnose and treat BCC also diagnose and treat SCC. We expect use will increase among the clinics that already have a device.

Revenue Stream

Our business model centers around digitizing dermatology (Fig. C7). By providing the dermatologist a real-time imaging modality and the pathologist with read-ready digital images, we believe we can drive earlier diagnosis and treatment of NMSC while providing economic efficiencies for the health care system. A cloud based digital hub will facilitate rapid sharing and interpretation of histopathology between dermatologists and pathologists. This digital hub will double as a data portal to advance our development and implementation of ML algorithms. We will collect the pathologists' annotations and diagnoses for the associated images (HIPPA compliant and de-identified) to further educate and improve our algorithms. These algorithms can augment interpretation by automatically segmenting areas of interest within images and streamline review of large datasets by flagging concerning images. As the volume increases through our hub, our algorithms will continue to improve, driving faster and possibly more accurate interpretations by the participating pathologists.

[redacted]

Figure C7. Business model and revenue streams based on flow of care (Fig. C3).

Revenue streams will come from three distinct sources: [redacted] Our revenue strategy leverages the high volume of NMSC visits and biopsies to build recurring revenue by utilizing a pay-per-use charge. Dermatologists will be charged by the [redacted]. This mirrors the reimbursement process of Medicare and private payers and is widely understood and well accepted by the dermatological community. We will [redacted] the MPM machines to dermatologists, which appeals to their purchasing habits and will inhibit underused devices in the field. Dermatology [redacted] will be rebated based on volume of use.

Table C4. Potential revenue with estimated 5% US market penetration.

	Total Addressable US Market	5% Market Penetration (Highest Volume Clinics)
Total number of clinics performing biopsies (estimated from 2015 CMS database)	6,500	325
# NMSC biopsies in US	10.4 million	2.1 million
Procedure Revenue	[redacted]	[redacted]
Lease Revenue	[redacted]	[redacted]
Pathologist Software-as-a-Service Revenue	[redacted]	[redacted]
Combined Annual Revenue	[redacted]	[redacted]

Clinicians can image as many lesions as is appropriate per patient visit, billing the CPT code we have targeted in each instance. We anticipate dermatologists will inspect at least as many suspicious lesions with our device as they do currently with biopsy, and that patients will seek clinics that offer our noninvasive alternative. With the prospect of increased patient traffic and favorable pricing of our systems, we believe there will be wide adoption of the EnSpectra System. We will price the use of our system such that the physician practice will financially benefit from the premium reimbursement rates for RCM imaging (see Table C2) and the cost savings of avoiding a biopsy (materials and nursing time related to tissue samples). Pathologists will be charged a monthly fee to use our digital hub to receive and review our images and to communicate their reports back to dermatologists. With a software-as-a-service model, we believe that monthly pricing can be volume based as well, depending upon the number of lesions reviewed per patient.

We have modeled a total available US market of over [redacted] (Table C4) following Steps 1, 2, and 3 laid out in "Market, Customer, and Competition" and "Production and Marketing Plan". A market penetration of 5% into the highest volume clinics translates into total annual revenues of [redacted].

August 03, 2020

Enspectra Health, Inc.
2490 Hospital Drive, Ste. 310
Mountain View, CA 94040-4125

Re: Grant Number: [redacted]

To Whom It May Concern,

This letter is to inform the NIH/NCI that EnSpectra Health has not received more than 15 SBIR Phase II awards from the Federal Government during the preceding five fiscal years.

Sincerely,

[redacted]

Gabriel N. Sanchez
CEO
[redacted]

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 02/28/2023

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ❍ Yes ● No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

❍ Yes ❍ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

❍ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? ❍ Yes ● No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ● Yes ❍ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ❍ Yes ● No

6. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 02/28/2023

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	SPECIFIC_AIMS.pdf
3. Research Strategy*	RESEARCH_STRATEGY.pdf
4. Progress Report Publication List	PROGRESSREPORTPUBLICATION.pdf
Other Research Plan Section	
5. Vertebrate Animals	
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	Combined_Letters_of_Support.pdf
10. Resource Sharing Plan(s)	
11. Authentication of Key Biological and/or Chemical Resources	
Appendix	
12. Appendix	

Funding Opportunity Number: RFA-CA-20-033. Received Date: 2020-08-30T20:48:47.000-04:00

SPECIFIC AIMS

Treating and diagnosing nonmelanoma skin cancer (NMSC) is a burden to patients, dermatologists, and payers across the US, costing [redacted] annually[1,2]. Incidence of NMSC accounts for over 75% of cancer in the United States[1,3], and continues to rise[4]. NMSC causes tissue destruction to skin and underlying tissue within months; each year left untreated doubles the surgical defect size[5]. Early NMSC lesions can be difficult to distinguish from temporary dysplastic or benign conditions. In these cases, the invasiveness of biopsy, which is currently required for gold standard pathology, can be a barrier to early diagnosis. A noninvasive method to detect NMSC, a long-sought goal, would allow for earlier diagnosis, reducing costs and morbidity. Noninvasive imaging of skin and skin cancer[6–10], including multiphoton microscopy (MPM)[11–18], has been an active area of research for over two decades, but solutions thus far have been too large, bulky, or expensive to be used regularly in the clinic or failed to resolve cellular features. *EnSpectra Health (EnSpectra) has invented a new approach to MPM that fits the needs of dermatologists and delivers both clinical value and usability*.

In Phase II, we successfully advanced development of a portable, skin-imaging microscope to be lighter, faster, and more robust. We also captured a library of hundreds of sections of various skin diseases, a subset of which was successfully interpreted by a cohort of 3 experienced dermato-pathologists and Mohs surgeons (blinded BCC evaluation with less than 6 hours of remote, online training: sensitivity, 94.6%; specificity: 88.0%, accuracy: 91.7%). We are on track to complete Phase II image interpretation, as well as gather and evaluate human performance data by the end of the project period [redacted]

Accelerated innovation across the lifetime of this project stems from our team's mastery of fiber delivery of ultrafast laser pulses, microelectromechanical systems (MEMS) based scanning, small beam waist optical engineering, silicon photomultipliers, and high frame rate oblique cross-sectional scanning which has allowed for unprecedented miniaturization, cost reduction, and imaging speeds within the field of high-resolution in vivo microscopy.

Specific Aims | Aim 1. Develop, manufacture, and test 8 portable, skin-imaging microscopes for commercial readiness (12 months). Phase IIB development will focus on ease of use in a clinical setting, design scalability, and accessibility of the MPM microscope design to support Aim 2's reimbursement clinical trial and prepare for scaled commercialization. The experiences of typical future device users (care providers) with the skin-imaging microscope will be systematically evaluated to inform and confirm design plans. New design innovation will include: 1) high-speed ambient light mitigation, 2) enhanced tissue contrast through spectral splitting, and 3) manufacturability of design. We will conduct formative human factors testing for these microscopes to improve the user interface and overall experience. This testing will also inform our design for better portability that we will incorporate into the commercial version. Aim 1 will culminate with the assembly of 8 commercial-ready systems to be used for internal testing and Aim 2's clinical trial. **Milestone.** Successfully implement design modifications to both satisfy human factors requirements (100% of 15 users make no critical use mistakes in second round of testing) and to prepare for scaled commercialization by advancing our device and documentation to a level of Clinical Verification and Validation Readiness.

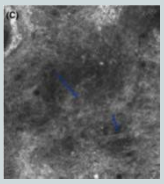
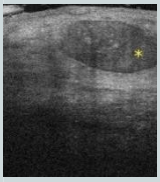
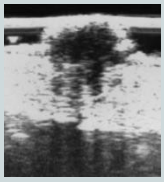
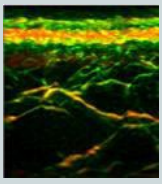
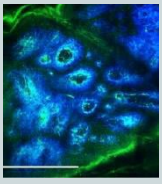
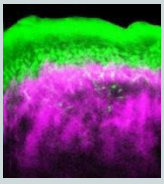
Aim 2. Conduct clinical trial to support reimbursement body of literature (24 months). We will prospectively gather in vivo EnSpectra device images of equivocal lesions of suspected BCC in 300 human patients at six clinical trial sites. The purpose of this study will be to determine diagnostic accuracy of physicians evaluating EnSpectra device images compared to gold standard histopathology slides in typical patients with suspected malignancy. **Milestone.** Demonstrate high sensitivity (>90%) and specificity (>90%) of in vivo BCC diagnosis to support payer reimbursement decisions and drive commercial adoption.

Impact. Advancement of MPM technology to a manufacturable and scalable design. Clinical data gathered from Phase IIB will represent an evidential tipping point for government and private insurance payers to support reimbursement of the Phase IIB product for use in evaluating BCC. This critical evidence will bolster confidence from clinicians, medical societies, and investors, accelerating commercialization and deployment of this technology to patients. Future and ongoing clinical studies, outside of the scope of Phase IIB, will methodically build a body of evidence to accelerate in vivo use and widespread reimbursement by payers of the MPM device with an eye toward revolutionizing the way medicine accesses histopathology of epithelial malignancies.

SIGNIFICANCE

Burden of nonmelanoma skin cancer | Nonmelanoma skin cancer (NMSC), is the most common form of cancer in the United States, and the incidence is rising[4]. Treatment of NMSC, which is usually surgical removal, is required to prevent tissue destruction and metastasis. Gold standard diagnosis of NMSC requires an invasive and destructive biopsy of the skin, which for the past 150 years, has remained the only clinically accessible procedure to image the subcellular structure of skin in order to detect cellular neoplasia. Diagnosis and treatment costs of NMSC are a burden, costing [redacted] per year[1,2].

Table R1. Technical performance characteristics of relevant high-resolution dermatological imaging methods compared with EnSpectra MPM (adapted[19]). *High definition OCT. †Oblique cross-section resolution.

	Reflectance confocal microscopy (RCM)[20]	Optical coherence tomography (OCT)[21]	High-frequency ultrasound (HFUS)[22]	Raster-scan optoacoustic mesoscopy (RSOM)[19]	Traditional multiphoton microscopy (MPM)[23]	EnSpectra MPM
Contrast mechanism	Light reflection	Reflection of low-coherent light	Reflection of ultrasound waves	Light absorption	Fluorescence and second harmonic generation	Fluorescence and second harmonic generation
Axial resolution, μm	3–5	5–10, 3*	30	5	1	3.6, 1.1†
Lateral resolution, μm	0.5–1	10–15, 3*	200	20	0.3	0.65
Penetration depth, mm	0.2–0.25	1–2, 0.5–0.57*	10	1.5–5	0.2	0.4
Typical field of view, mm^2	0.5 × 0.5	6 × 6 1.8 × 1.5*	8 × 12	4 × 2	0.35 × 0.35	0.4 × 0.4
Representative in vivo image, within 5 years						
Diagnostic potential	Horizontal scanning and no color contrast limit future potential	Low resolution prevents diagnostic potential	Low resolution prevents diagnostic potential	Low resolution prevents diagnostic potential	Slow speed & immobility limit surveying, increased risk of false negative	High diagnostic potential

Although millions of biopsies to rule out NMSC are performed annually in the United States[1], the true burden of NMSC lies in the diagnoses that are not made soon enough. For every year left untreated, ultimate NMSC defect size will double[5]. The increase in lesion and defect size caused by delays in treatment leads to a substantial increase in morbidity for patients and much greater costs to the healthcare system. Delay in diagnosis and treatment is caused in part by patient delay in seeking medical care, but also by the bland clinical appearance of early NMSC disease that fails to warrant invasive biopsy. In these cases, the small lesions are clinically monitored for months and sometimes years to avoid unnecessary biopsies. *Noninvasive pathologic evaluation of skin is an important unmet need that would reduce the barrier to early diagnosis of dangerous, destructive, and costly NMSCs.*

Other attempts | The need to improve early detection of NMSC is well-known and has created a robust field of innovation and interest. Three major approaches exist: (1) Low resolution imaging modalities, sometimes paired with digital analysis, to assist with screening, such as superficial photography [24] and dermoscopy[25], which are not likely to become diagnostic, (2) digital pathology of scanned, hematoxylin and eosin (H&E)

stained slides[26], which improves access to digital images of pathology, but does not impact the time delay caused by biopsy invasiveness, and (3) noninvasive high-resolution dermatologic imaging methods, which have generated much excitement for their ability to better identify skin cancer without taking a biopsy (Table R1). Thus far, however, these noninvasive imaging modalities have suffered from limitations that have likely impeded their diagnostic potential (Table R1).

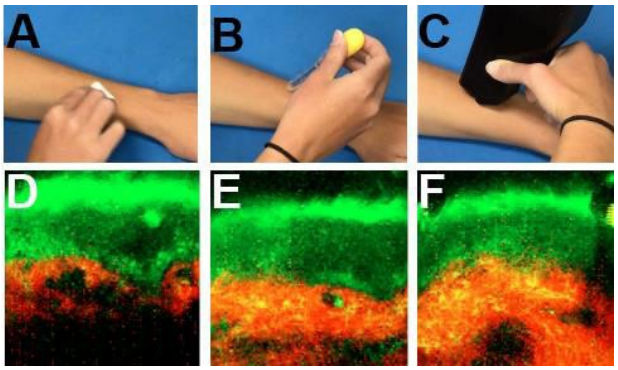


Figure R1. Still images within video highlight EnSpectra's innovation of 9.4 fps Rapid Roam cross-sectional imaging, at a lower resolution, which allows for precise targeting of features-of-interest before capturing high resolution snapshots (not shown due to video size limit). Skin preparation is accomplished in seconds, which includes (a) a swab of the skin with alcohol and (b) a drop of glycerine. (c) The microscope can be moved across the skin by hand with real-time image feedback. Examples of Rapid Roam (0.11s capture) in skin of the (d) forearm, (e) forehead, and (f) nose.

Multiphoton microscopy of skin | Multiphoton microscopy (MPM) of skin for the identification of skin cancer has been extensively studied, both ex vivo and in vivo[11,12,14,15]. MPM, as developed by JenLab GMBH, has even received the CE Mark of regulatory approval for clinical use[27]. Because of this prior research, we know MPM can provide diagnostic information about NMSC and the ideal excitation and emission spectra for this application. This early work guided our Phase I and II applications and subsequent and ongoing success with completing our Phase I and II aims. Despite research and development by other groups, MPM has not achieved the form factor required for widespread clinical adoption. In Phase I and II of our proposal we sought to demonstrate feasibility of a highly miniaturized MPM system for rapidly imaging skin and build compelling evidence for use in evaluating skin cancer. Our device overcomes the limitations of other embodiments of high-resolution approaches to noninvasive imaging (Fig. R1).

INNOVATION

Progress Report for Phase II | Substantial innovations and program advancements were accomplished during the Phase II portion of this project. Overall, we sought to build evidence of robust and information-rich imaging and advance EnSpectra Health's portable, skin-imaging multiphoton microscope toward FDA clearance. Here, we report the on-track completion of Phase II Aims.

Progress Report Phase II Aim 1 | Advance development of portable, skin-imaging microscope for clinical trial.

Importance. The design specifications of the microscope were guided by the needs of practicing dermatologists across the United States. In short, these dermatologists requested a device that provided high-quality and familiar images of skin layers in cross-section. *In contrast to the Phase I device, they requested a device that was easier to use, faster, and smaller.* The device that we built during Phase II, therefore, is well-suited to meeting the clinical needs of typical dermatologists and their patients. ***Progress.*** We successfully constructed the portable MPM microscope for imaging skin and exceeded the innovations we proposed in Phase II: robust cable management, optimization of the imaging cap, software instructions, intuitive device alignment with the skin, hardware customization, and software refactoring. Innovation beyond the original Phase II proposal centers on the implementation of new solid-state multi-pixel photon counter (MPPC) sensors enabling faster

Table R2. Optical performance of the Phase II technology.

Feature	Measured performance
Horizontal resolution (FWHM)	0.65 ±0.02 μm
Axial resolution (FWHM)	3.59 ± 0.29 μm
Oblique cross-section resolution (FWHM, 35° from axial)	1.26 ± 0.07 μm
Depth in vivo	405 μm
Field-of-view	405 μm
In vivo oblique 10%-90% edge response	50 μm depth: 1.05 ± 0.13 μm 300 μm depth: 1.84 ± 0.27 μm

scanning, simpler electronics, and new approaches to imaging in ambient light conditions. The use of the device is demonstrated in the included video and Figure R1, which also highlights a new innovation of Rapid Roam. To our knowledge, we are the first to achieve live skin imaging with these sensors.

Phase II Aim 1a. Create design controls to minimize risk of user errors. ***Importance.*** The FDA has increasingly emphasized the role of human factors in the performance of medical devices because the way a device is used by care providers determines the safety and effectiveness of the device.
Progress. We executed a series of human-factors inspired design advancements to better match clinical practice. *Cable management*. An intuitive cable, wand, and briefcase system were developed to reduce the risk of twist and crush damage. The length of the cable, which also impacts laser performance, was also optimized to be shorter (1.2 m). *Silicone cover*. In our Phase I and Phase II projects, we have successfully used a disposable, silicone cap paired with glycerin to create a simple high friction interface with skin that stabilizes images and eliminates motion artifacts. In Phase II, we executed a human-factors design to prevent the formation of an air bubble between the cap and the terminal glass lens that occurs when the user does not fully engage the cover. To mitigate this issue, we modified the molded silicone cover with a concave surface (Fig. R2a). We also incorporated the use of adhesive packaging of the silicone cover to eliminate the need for cover cleaning. *Software instructions*. We have incorporated instructions directly in the software interface of the device to limit the need for separate file-based Instructions for Use (IFU), which can be easily misplaced or overlooked. As needed instructions are generated through real world use, software-based instructions will become a valuable platform for development.

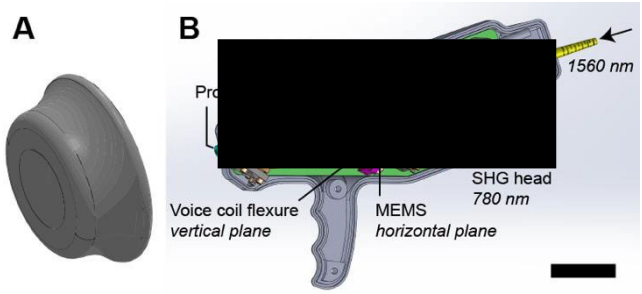


Figure R2. Handheld wand with silicone cover. (a) Silicone cover with concave contact surface. (b) Computer-aided design rendering of Phase II wand design. Scale bar: (a) 5 mm, (b) 50 mm.

Imaging normal to the skin. During early testing of our Phase I imaging wand, we learned that form factor and ergonomics are important factors for successful imaging. The architecture of our Phase I imaging wand made it difficult for users to intuitively align the imaging objective perpendicular to the surface of the skin in vivo, which is required for full-depth images. To define the shell geometry shown in Figure R2b, we developed five different possible wand geometries for our Phase II prototype and created foam mock-ups for user testing. We systematically tested the wand geometries with eighteen dermatology care providers (including key opinion leaders from teaching institutions across the country in addition to local mainstream dermatologists) on criteria such as ability to target regions of the face, intuitiveness of orientation, and overall comfort and "hand-feel" (see HF int. test 1 in Figure R3). The current shell geometry was the most popular and was either the first or second choice of 90% of the providers. We carried this geometry forward for our Phase II wand, adapting the optical pathway to accommodate the desired form factor (Fig. R2b).
Changes. We report no variations to Aim 1a from the original Phase II application.

Phase II Aim 1b. Customize hardware and refactor software to create a smaller, faster, and more robust system.
Importance. Dermatology clinics are fast paced environments that require efficient and intuitive tools to provide effective care for the maximum number of patients. Our product's size, mobility, and speed of image acquisition are essential factors to consider for widespread clinical adoption.
Progress. (see "HF int. test 2 in Figure R3). *Reduced size and mass for clinical operation*. Our human-factors inspired design activities provided critical information about the product size and ergonomics that would be necessary for efficient clinical use. We adapted our optical pathway to the clinician preferred geometry, successfully accommodating the desired form factor by folding the optical path with a series of reflective mirrors in custom designed steering mounts (Fig. R2b). At the laser output where we required additional alignment degrees of freedom, we utilized a swiveling optical window to displace the beam without the need for an additional mirror. Within the briefcase, we implemented a custom streamlined control board using a combination field-programable gate array (FPGA) and microcontroller architecture. Implementing light sensing with MPPC sensors greatly reduced power supply and analog sensing complexity, further streamlining our electronics components. In our new architecture, the combined mass of our wand, laser, collection module, electronics board, and electrical cables is only 2.8 kg. We will integrate these modules with a 1.8 kg medical grade tablet PC and enclosure with an estimated additional mass of 2kg, bringing the fully integrated system

under our target mass of 8 kg. *Increased image acquisition speed.* In our Phase II proposal, we targeted a maximum frame rate greater than 4 frames/s. Our improved wand features two imaging modes: a Rapid Roam mode of 9.4 frames/second, and a high-resolution capture of 1.2 frames/s. We achieved these speeds by optimizing the drive waveform of our microelectromechanical systems (MEMS) scanner to boost our line scan rate. We maintained image quality at these faster rates thanks to the improved bandwidth and noise characteristics of our new MPPC sensors, and faster data acquisition with our FPGA based architecture.

Changes. We report no variations to Aim 1b from the original Phase II application.

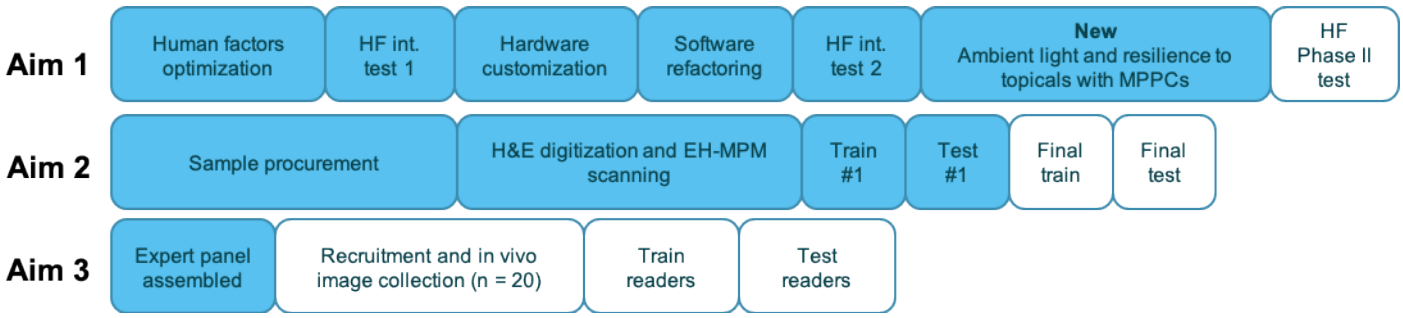


Figure R3. EnSpectra is on track to complete Aims 1, 2, and 3 by the end of the project period [redacted] Aim 1) We have completed development for human factors (HF) optimization, hardware customization, software refactoring, and the newly added resilience to ambient light and topical cosmetic products. We have also conducted 2 intermediate (int.) user tests. Final HF Phase II test will be completed in [redacted]. Aim 2) Full scale sample procurement, digitization, imaging, and preliminary testing were successfully completed. Final training and testing will be completed by [redacted]. Aim 3) EnSpectra is on track to conduct an in-house Skin Structure and Function study in the [redacted] to provide a first tier of evidence for a 510(k) submission [redacted].

**NEW* Phase II Aim 1c. Fully integrate Multi-Pixel Photon Counters to provide greater resilience to ambient light and skin topicals, improve imaging speed, and streamline system architecture.*

Importance. *Imaging in ambient light*. In typical clinical practice, dermatologists in contrast to radiologists work in brightly lit offices to better see human skin at the macroscopic level.

Interference of topical cosmetic products. We noted anecdotally during Phase I in vivo testing in a diverse set of research subjects that topical cosmetic products persisted on the skin even after cleaning with an alcohol prep pad. Microscopic cosmetic particles on and within the skin folds are frequently fluorescent and interfere with imaging by leaving saturated streaks across the image. Improving imaging of skin despite the presence of topical cosmetic products will improve the accessibility of EnSpectra's skin-imaging device for point-of-care use in patients who use these cosmetic topicals without requiring a lengthy skin-cleaning process.

Improving imaging speed and streamlining system architecture. Reducing system complexity will lead to reduced cost of production and improved manufacturability, boosting the likelihood of successful commercialization and reliable product performance.

Progress. To simultaneously address the challenges with ambient light and the interference of topical cosmetic products, we advanced the design of EnSpectra's skin-imaging microscope to sense light with integrated MPPC modules instead of the PMTs used in our previous design. The high bandwidth of the MPPCs eliminated streaking from topical particles that resulted from slow decay of the PMT transimpedance amplifiers. The large dynamic range of the MPPCs also enabled subtraction of the background ambient signal to recover the tissue image when operating in normal room lighting. The MPPC modules feature advantages over PMTs that simplify implementation and improve reliability. MPPCs run on low voltage and are completely resilient to overexposure. The modules have internal transimpedance amplification so simple voltage amplification is all that is needed to capture images. The modules are also a fraction of the cost of PMTs, with much shorter lead times and can be purchased in larger quantities. We have fully and successfully integrated MPPCs into working prototype of the device, which was used for all Aim 2 and Aim 3 images.

Changes. While we have been interested in solid state sensors for some time, we had planned to transition away from PMTs in the next generation of our product. The motivation to develop this innovation in our Phase II project arose due to the growing Covid-19 pandemic. In the early stages of the pandemic, it became clear that trial activities in clinical settings would stall and we would be unable to access patients for in vivo testing. Rather than hibernate and suffer future delays, we chose to complete this product initiative now. Clinical trials

are still stalled across the country, but by already completing the transition to MPPCs we can stay on our commercialization timeline once trials resume.

Progress Report Phase II Aim 2 | Develop library of slide images of targeted skin diseases comparing MPM to traditional histology.

Importance.

Progress. We collected 396 paired H&E-stained and unstained sets of slides of 132 independent skin samples (Table R3). Ten samples from Hispanic and black patients are included to ensure EnSpectra imaging performs as expected when skin is pigmented. We commit to continuing to search for samples of skin malignancy in patients with darker skin, although rare, in this and future Phases of this project. EnSpectra MPM images of BCC and normal skin were presented to blinded readers for interpretation (n = 31 BCC; n = 25 normal). Readers successfully identified presence and absence of BCC in this initial analysis (sensitivity, 94.6%; specificity: 88.0%, accuracy: 91.7%). To complete Aim 2 as planned, we will challenge a new set of blinded readers with the complete set of skin samples with 30% of the samples reserved for training, that include benign and malignant skin conditions commonly included in the BCC differential such as melanocytic nevi and SCC in addition to normal skin.

Table R3. Phase II Aim 2 Slide Atlas samples.

Condition	Sample #
BCC	54
Normal	
- Hispanic or black	10
- white	15
Not BCC nor normal	53
Total	132

Changes. We report no variations to Aim 2 from the original Phase II application.

Innovation. *Adaptation to protect human health*. The emergence of the novel coronavirus pandemic around the time of completing Aim 2 inspired our team to adapt plans quickly and methodically to achieve progress without risking the health of our team or colleagues. We instituted the following measures: (1) temporarily rented private office and lab space to limit access, (2) rotated employee shifts to limit overlap of employee presence in the lab, (3) all employees work from home whenever possible, which included building the slide scanning apparatus from home-based workshops, and (4) implemented a *fully remote, blinded reader online training and testing paradigm* in some cases with physician colleagues we have never met face-to-face. We had not originally planned for a fully remote train-and-test protocol, but its successful execution speaks to the world's increasing need for fully digital access to histopathology.

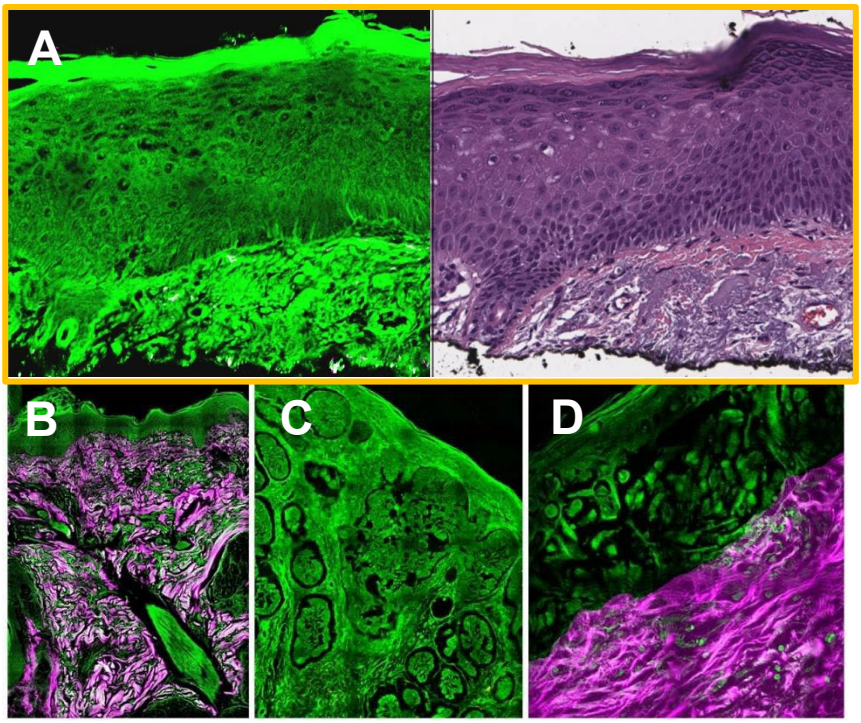


Figure R4. Ex vivo imaging of unstained skin samples with fully automated scanner. (a) Screenshot of *Helios* software showing EnSpectra (left, unstained) and H&E (right, stained) paired slides for feature comparison by clinicians. Example whole-slide mosaics of: (b) Healthy skin, (c) Basal cell carcinoma, and (d) Melanoma.

Visualization software for paired slide comparisons. One of the challenges of remote training and review is sharing large, high resolution images in an accessible format for clinicians. Much like existing slide scanning companies, we felt the need to develop a custom image visualizer, *Helios*, to permit online viewing and comparison of paired MPM and H&E slide images (Fig. R4a). This software coordinates panning and zooming of paired images from our MPM scanned slides and matching scanned H&E slides (Leica Biosystems, Aperio).

Fully automated whole slide imaging scanner. To further protect our employees, we created a fully automated slide scanner that minimizes the need for in person operation. Our system only requires human interaction to switch samples and set the scan coordinates. Our custom GUI synchronizes the image capture from our vertically mounted wand with the X-Y stage movements in a pattern that facilitates automated stitching. The scanner sends text alerts when it is completed and ready for the next sample. We believe this scanner, along

with our *Helios* visualizer, is the forerunner of a wholly new Enspectra Health product for *ex vivo* bedside histopathology and cytology in intraoperative and office settings.

Progress Report Phase II Aim 3 | Conduct in vivo human performance testing to support FDA clearance.

Importance. The requirement of high quality clinical data demonstrating safety and effectiveness of EnSpectra's MPM imaging has not changed despite the challenges that now exist with patient recruitment due to the global pandemic. If anything, the challenges of the present pandemic have highlighted the need for the existence of our technology. Now more than ever, clinicians need tools that allow rapid turn around and sharing of data to detect skin cancer in patients with decreased mobility and medical access. We are witnessing a positive shift in clinical attitudes in favor of new digital health technologies that will likely persist after the pandemic.

Progress. *Although no subjects have been recruited for Aim 3, we have gathered in vivo images using the advanced MPM device developed in Aim 1 (Figs. R1 and R6). We will be ready to recruit and image subjects as soon as conditions permit.*

Changes. *Adaptation to protect human health of potential cancer research subjects*. The ongoing global COVID-19 pandemic has dramatically impacted both clinical trial recruitment and the practice of dermatology. During the pandemic, skin cancer diagnoses have decreased by nearly 70% in the United Kingdom[28], and our colleagues in the United States have confirmed a comparable reduction in patient visits. Unfortunately, the most prevalent dermatology patients are also at risk of serious harm from COVID-19 disease.

Conducting a large scale clinical trial with these patients before coronavirus is controlled would be both financially inefficient due to slow recruitment and medically irresponsible due to patient risk. To adapt to this reality, we implemented Aim 1c to continue accelerating the development and innovation of the EnSpectra MPM imaging and redesigned the in vivo human performance study to be smaller and involve healthy subjects only (n = 20) with an endpoint of interrater reliability of skin structures (Cohen's $\kappa > 0.75$). The necessary study described in the Phase II application for FDA clearance will still be completed, but at a later time that coincides with the return of typical patients to dermatology practices, and will be financed with third-party funds (see letters of support).

Innovation within Phases I and II

In the previous section, we described what we achieved relative to the Phase II Specific Aims. Here, we describe how these innovations are unique and how we achieved them through unconventional approaches.

Improving resolution and light collection by increasing numerical aperture (NA) is one of the most fundamental principles in microscopy. Optimizing for higher NA is taken as a given in nearly every major imaging modality today, especially when axial imaging is a goal as is the case for in vivo skin imaging. JenLab and other imaging groups have used commercial objectives, such as from Olympus or Zeiss, with NAs > 1.0[13,23]. Unfortunately, these objectives have large entrance pupils and mechanical dimensions that prevent miniaturization. High NA objectives also return wide cones of signal light that necessitate bulky collection arrangements directly behind the objective, further increasing size. In Phase II of this project, we advanced development of in vivo imaging device based entirely around small beam waist (SBW) optics with moderate NA (~0.7) that nonetheless matches the performance of larger systems with NA> 1.0. Our approach puts us at odds with the standard ethos of optical design, however we report that SBW optics paired with MPM enable substantial advantages and innovations that are not possible with traditional approaches.

Innovation for high resolution cross-section scanning

Imaging the skin rapidly in cross-section is an important feature for practicing dermatologists and dermato-pathologists. Although one other group has reported cross-section MPM imaging[13], the reported system images less than 25% of the FOV we achieved in Phases I and II and requires over 30 s to capture a single image whereas we capture an image in as little as 0.11 s in roaming mode.

One of the primary challenges with imaging in cross-section is axial resolution. Both lateral and axial resolution improve with increased NA, but axial resolution depends more strongly on NA. Assessing histopathology requires an image resolution near 1.0 μm to visualize cells and nuclei. At our wavelength of 780 nm, this resolution is possible in the lateral direction with an NA near 0.5 but requires an NA >1.0 in the axial direction. Increasing NA above 1.0 becomes increasingly challenging when balancing FOV, off-axis aberrations, probe tip diameter, and signal collection for sensing.

Our device can simultaneously translate the optical focus within the tissue in the X, Y, and Z directions. By coordinating these movements, we developed a method to scan through the tissue in an oblique imaging plane to create an image from an angled cross-section of the point spread function (PSF). Because the three-dimensional PSF is essentially an elongated ellipsoid, even at modest oblique angles, the effective PSF dimension is greatly reduced compared to pure axial (XZ) imaging (Fig. R5). We achieved near 1.0 µm resolution in cross-section with an NA of ~ 0.7 when scanning in a plane approximately 35° from vertical (Table R2). Imaging depth is not altered by oblique scanning (Fig. R5b). All in vivo cross-section images in this grant were scanned obliquely at 35°.

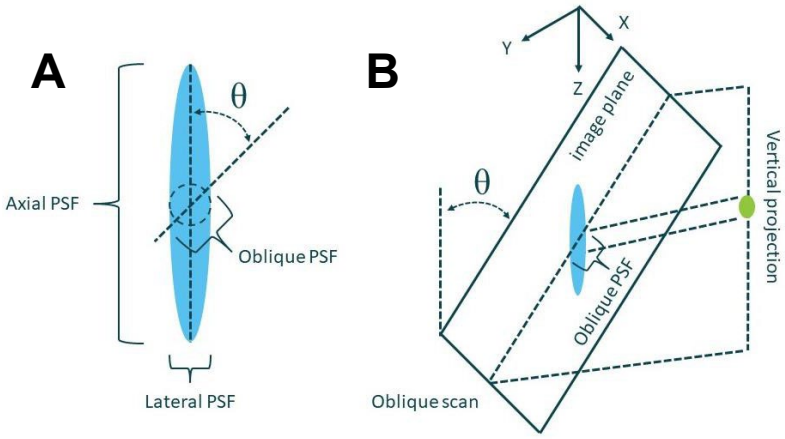


Figure R5. Oblique cross-sectional imaging. (a) 3-Dimensional point spread function (PSF). (b) Oblique imaging plane transects the 3D PSF, creating an effectively smaller PSF. Oblique plane projects to the same Z-depth in the vertical direction.

Oblique scanning greatly improved in vivo resolution (Fig. R6). Cellular nuclei, ~ 5 µm diameter, are readily visible in our images and provide important histologic information. While we achieved our resolution goals under ideal conditions (Table R2), it is important to estimate the actual resolution achieved in vivo throughout the depth of imaging. In our images, bright cells in the basal layer and sparse cells in the dermis produce signal from the cytoplasm that contrasts strongly with regions of little to no signal. We analyzed the intensity profiles across these edge transitions and estimated the in vivo resolution from the 10-90% edge response at 1.05±0.13 µm at a 50 µm depth and 1.84±0.26 µm at a 300 µm depth (Figs. R6B, averages of n = 5 edge profiles, respectively). We scanned approximately half the typical field of view to achieve finer sampling for the edge response measurement. Based on reviewer feedback from our Phase I progress report, we were careful to ensure the measured edge responses were from features in the images that were not saturated. Our measured edge responses are consistent with features present in our images, such as intercellular spaces and fine elastin fibers, that are smaller than the 2µm reported axial resolution of the Jenlab MPTFlex (Fig. R6C). It is unclear if the reported MPTFlex resolution was measured under ideal or in vivo conditions. Regardless, exceeding the MPTFlex resolution during freehand in vivo imaging is a remarkable achievement and is a substantial innovation for the field of MPM.

Feedback from dermatologists reinforced the advantage of having a cross-sectional view to see the various skin layers in relation to each other. While imaging the vertical plane of skin accomplishes this objective, so does imaging in an oblique plane. In fact, dermatologists acknowledged that traditional cut histology sections are never perfectly normal to the skin and praised our oblique sections for facilitating interpretation.

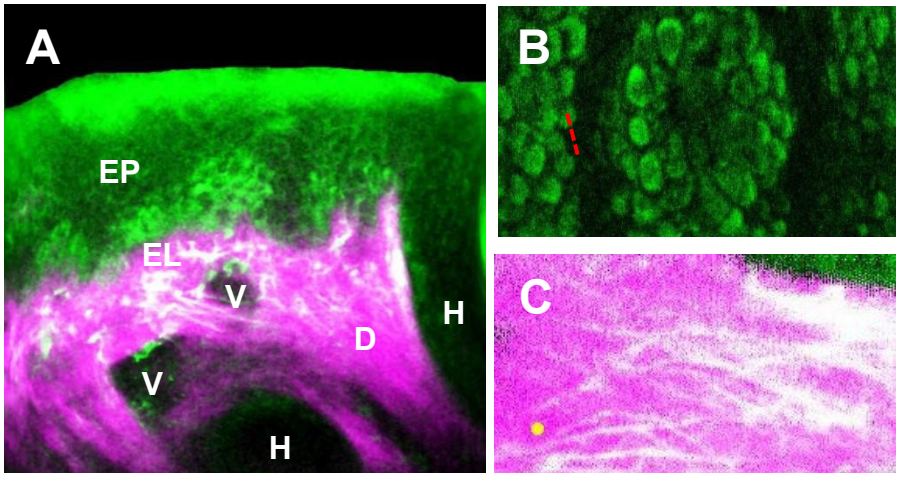


Figure R6. Cross-sectional, freehand imaging in live human skin. (a) Real-time cross section of the forehead (0.85s capture). Green: 2PF, magenta: SHG. (b) Magnified view via smaller scan amplitude of basal cells in the forearm. Dashed red line indicates location of edge response measurement. (c) Digitally magnified view of fine elastin structures in the dermis of the nose. Yellow circle is 2 µm in diameter. EP: epidermis, D: dermis, V: vessel, EL: elastin, H: hair follicle.

Innovation for limited motion artifacts

In vivo MPM imaging is plagued by difficulties with motion artifacts due to breathing and other small movements. Understandably, reviewers of our Phase I application were concerned with the risk of motion artifact. A core innovation of our technology—remote z-scanning—creates an advantage that overcomes this important obstacle. Unlike other systems that scan through tissue depth (z-scanning) by

varying a gap between the terminal lens and the tissue, our system z-scans remotely within the microscope while the probe tip remains in static contact with the skin. Without a gap, there is no relative motion between the imaged tissue and our optical pathway, eliminating the need for rigid fixation to the skin or the use of elaborate movement mechanisms.

Simple friction dramatically minimized motion artifacts. We optimized the high-friction interface by using a terminal silicone spacer with high optical clarity combined with glycerin as an immersion fluid. In our Phase I prototype (4s/frame), we assessed motion artifacts during freehand imaging in live skin by continuously streaming images in once subject for 20 minutes (290 images) and tallied the frequency of motion artifacts. We found artifacts in 36 of the 290 images, or about 12.4%.

Innovation for Rapid Roam mode and further reduction of motion artifacts

In our Phase II device, motion artifacts were all but eliminated by adopting a dual imaging mode with high-speed roaming (9.4 frames/s) and high-resolution capture (1.2 frames/s). Our MEMS mirror scans lines sinusoidally in both directions at 600 Hz (single line frequency of 1200 Hz), completing each line in 0.85 ms. We oversample our lines at 16 MHz and digitally integrate our signals during the full pixel interval to maximize our signal-to-noise ratio. The roaming mode (128 pixels x 128 lines) offers a compromise between speed and resolution: imaging eight times faster but with one eighth the resolution of the high-resolution capture (1024 pixels x 1024 lines). The roaming mode is highly responsive and helps users identify regions of interest for a high-resolution capture. Even with reduced resolution, it is possible to resolve individual cells, observe the layers of the epidermis and dermis, and other key features such as hair follicles, blood vessels, and ducts. Once the device is positioned over the region of interest, the user can capture a high-resolution image in under a second, over four times faster than our Phase I device. Thanks to higher bandwidth and improved noise characteristics of our new MPPC sensors, images from our Phase II device are comparable to our Phase I device despite almost five times faster imaging.

Miniaturization enabled by small beam waist optics

In our Phase II MPM device, our optical beam waist within the imaging wand never exceeds [redacted] in diameter. Imaging with moderate NA and a custom designed objective with an entrance pupil of only [redacted] removes the need for excessive beam expansion. The lenses in our system operate primarily in the paraxial region, minimizing aberrations and improving off axis resolution. With smaller mirrors, lenses, and actuators, scanning speeds are faster and the axial translation of our remote z-scanning becomes much shorter. These miniaturization innovations enabled by SBW optics quickly compound. A related in vivo MPM imaging system (MPTFlex developed by JenLab) is advertised as “portable”. However, the MPTFlex is 250 kg (710 cm x 960 cm x 1,400 cm), about the mass of a large upright piano, and requires a gantry arm to position the imager. In contrast, our Phase I device [redacted] imaging wand freely moves at the end of a flexible cable.

A key advantage of SBW optics is lateral scanning with a small diameter MEMS mirror instead of a galvanometer. Traditional MPM systems typically utilize paired galvanometers for laser scanning (typical footprint 100 x 100 x 50 mm). Drawbacks of galvanometer pairs include a large mechanical footprint and the presence of a gap between mirrors that causes shifts in the optical axis during scanning. Unlike paired galvanometers, our single MEMS mirror rotates along two intersecting axes that keep the optical path concentric with the mirror regardless of scan angle and enables our z-scanning approach that alters the convergence or divergence of the beam ahead of the MEMS mirror via a voice coil actuator. Additionally, our MEMS scanner is very small [redacted] and delivers a fast frame rate [redacted] due to its small mass.

Our system utilizes a small fiber laser [redacted] with low power requirements [redacted]. Using a fiber laser provides many advantages: a robustly aligned system necessary for portability, device flexibility (cable vs gantry arm), and improved light transmission from laser to tissue. A common challenge with fiber delivery of ultrafast pulses is pulse broadening due to dispersion. To overcome this challenge, we used a 1560 nm fiber laser with an ultra-small PPLN frequency doubler in the handheld microscope itself to create 780 nm light for MPM. We collaborated with [redacted] to design this PPLN module [redacted] which is a miniaturized version of their standard product. We provided performance testing, specifications, and dispersion characteristics for the optics in our handheld microscope. The [redacted] laser further features a tunable range that trades off pulse width and energy. We optimized the tunable range with our integrated system, ultimately achieving a pulse width below [redacted] fs.

Innovation for cross platform use: bedside to bench to algorithm augmented diagnoses

We developed our fully automated scanner to provide a safe means to benchmark our MPM system against traditional H&E staining during this Covid-19 pandemic. We believe we have indirectly created a system that paves the way for clinical applications where immediate microscopic analysis of freshly sampled tissue or cells is needed. Although our current system images unstained slides, with modification it would be possible to image freshly excised tissue with no need for staining. We are aware of a similar but much larger system in the research community that still requires tissue staining[29]. While the size of this system is not reported, we know that the laser system alone is over 100 kg. Our ultra-portable stain-free system could be deployed in a surgical or office setting with minimal disruption.

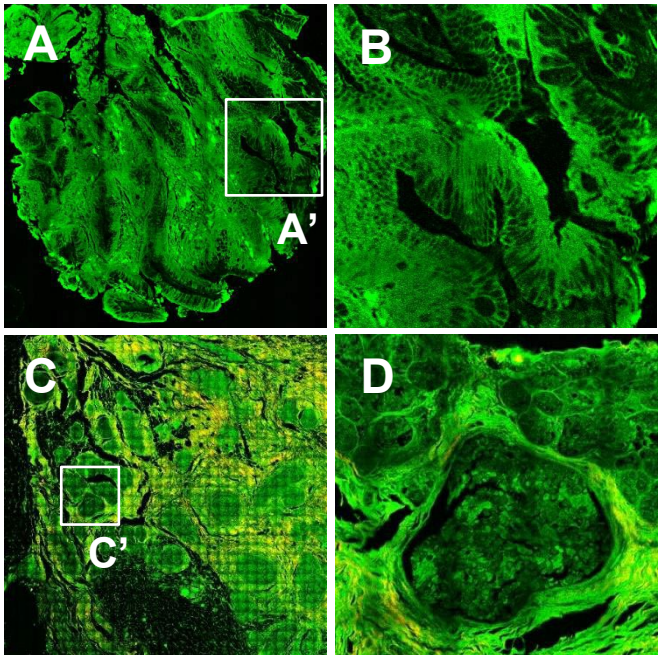


Figure R7. Tissue feasibility testing with whole slide imager. (a) Unstained sample of Barrett's esophagus. (b) Magnified view of region a'. (c) Unstained sample of breast cancer. (d) Magnified view of region c'.

We envision applications, such as surgical removal of malignant tumors, where immediate analysis of tissue histopathology could lead to more effective removal of tumors while sparing healthy tissue. In an office setting, freshly collected cytology samples (e.g. from cervical swabs), could be applied to slides and directly imaged in the office for immediate results.

One of the greatest advantages of our current scanning system is that we can explore the imaging characteristics on various tissues of interest by simply imaging unstained slides. We have already imaged breast and esophageal cancers with our system (Fig. R7). We plan to investigate multiple tissues for compelling future imaging applications to build confidence and evidence that our device will provide clinical value.

Our scanner generates massive amounts of data ideal for preliminary ML algorithm development. The samples imaged for this project alone yielded 241,694 classifiable image tiles of skin samples. A key advantage of our scanner is that we can prototype algorithms using unstained slides before committing to the cost and expense of building in vivo datasets. We will use our ex vivo datasets to prototype different algorithms and determine which are most likely to be successful in vivo. We will also use ex vivo data sets to initialize parameters for more efficient training of algorithms.

Innovation for inexpensive and reliable photon sensing using MPPCs

In our Phase II project, we added the new innovation of implementing solid-state MPPC sensors in lieu of traditional PMTs. We believe multiphoton microscopy will benefit from the reliability, reduced cost, and superior performance of solid-state components. Using our Phase I device, we characterized the light levels of our biologic signals and performed modeling that suggested that MPPCs would provide superior signal-to-noise performance for our application. We found the benefit was even greater than predicted because the amplified power supplies for the PMT modules coupled substantial additional noise into our images.

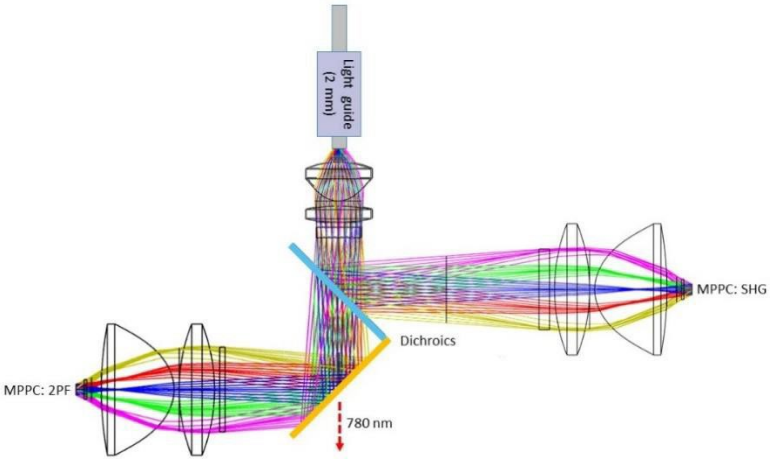


Figure R8. Optical pathway for MPPC sensing. Leakage 780 nm light from laser passes through to minimize background.

We used a pair of cooled MPPC modules with built in current conversion and good sensitivity from 390-650nm to record second harmonic generation (SHG) signals on one channel, and two-photon autofluorescence (2PF) signals on the other (Fig. R8). We used high speed voltage amplifiers to match the input into our analog to digital converters and take advantage of the superior bandwidth of the MPPCs. This addition eliminated streaking and smearing of saturated pixels in our images that we experienced with our PMTs. In our Phase IIB

project, we will develop a 3-channel sensing version to further split the 2PF signal and provide additional tissue contrast (see Approach Aim 1).

One of the challenges with MPPCs is a smaller active area compared to most PMTs (typically 3mm or less). Our SBW design was critical for efficient delivery of light to these new sensors. The pupil of our objective is only 1.3 mm in diameter and permits efficient collection of light into a 2mm diameter liquid light guide. We designed a custom optical configuration to efficiently image the collected light within the MPPC active area (Fig. R8).

Innovation for imaging in ambient conditions

Our new MPPC sensors boast a dynamic range of nearly five orders of magnitude. This range is sufficient to record not only our biological signals, but also the ambient light that leaks through the skin under normal light conditions and couples into our optical pathway. We developed an approach to subtract the ambient background in real time and recover only the biologic MPM signals, permitting imaging under normal light conditions. When scanning through the skin, there is a region where the excitation light is still within the clear silicone cap which contributes no MPM signal. Signal recorded in this region must be ambient leakage. Since the cap is in a completely predictable location, we average the ambient light in this region on both channels and subtract that value from each successive measurement for the reconstructed image. This method effectively eliminated the mean background leakage and is adaptable to changing light conditions (Fig. R9). The only drawback is an inability to cancel dynamic ambient fluctuations that can still interfere with imaging. In our Phase IIB project, we will implement a faster method utilizing a beam block at the edge of the fast axis scan. This will effectively shutter the laser at the end of each line, leaving only the ambient leakage which we can measure and cancel at our line scan frequency of 600Hz.

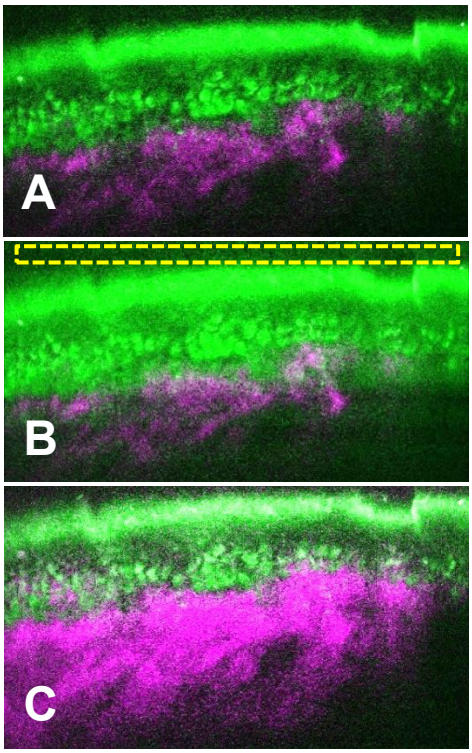


Figure R9. Ambient light mitigation with background subtraction. (a) Imaging with lights off. (b) Lights turned on. Notice saturation and bands of fluctuating ambient levels (c) In vivo imaging with ambient cancellation enabled. Images are brightened to highlight the cancellation effect. Dashed box is the cap region.

APPROACH

Phase IIB Aim 1 | Develop, manufacture, and test 8 portable, skin-imaging microscopes for commercial readiness (12 months).

Aim 1 Rationale: We have made rapid progress proving the feasibility of in vivo skin imaging with portable multiphoton microscopy. We are now prepared to advance our technology to a level commensurate with acquiring human clinical data in support of our regulatory, reimbursement, and early commercialization needs. We will continue to innovate and create new scientific advances with the goal of translating our discoveries into a reproduceable and manufacturable architecture that is safe for patients and users.

Aim 1 Methods:

Prior to manufacturing our 8 systems, we will work to finalize key innovations and achieve a design freeze. Two areas of continued innovation from our Phase II project are ambient light mitigation and spectral splitting of our 2PF signal. We proved the feasibility of ambient light subtraction using MPPC sensors in our Phase II prototype. In this first iteration, we were only able to reject ambient light fluctuations up to approximately 1 Hz, leaving us susceptible to higher frequencies. In Phase IIB, we will integrate a beam block in a conjugate plane within our imaging wand at the edge of the scan field. By slightly over-scanning into this block and effectively shuttering the laser, the edges of our image will contain only ambient background and no biologic signals. We will record the ambient light in this region on both channels and subtract that value from each successive measurement for the reconstructed image. This approach will allow us to cancel ambient fluctuations up to 600 Hz (Nyquist) and will be inherently synchronized with the MEMs scanning motion. We will crop the cancelation region out of the final image.

In our Phase I and Phase II devices, we provided visual contrast between cellular tissues and stroma by isolating SHG and 2PF signals on separate MPPC sensors (Fig. R8). While building our Phase II slide library, we noticed that some samples produced very little SHG in the dermis. This was particularly prominent in

highly sun damaged samples which also tended to contain skin cancer (Figs. R4a and R4c). We suspect sun exposure diminishes SHG signals by damaging collagen while inducing an increased deposition of elastin (solar elastosis). Presently, our single 2PF sensing channel combines signals from NADH (cellular cytoplasm), keratin, elastin, and melanin. The only visible differences in tissue from these signals are image brightness and morphology. In our Phase IIB project, we will implement a 3-channel architecture by splitting the 2PF signal into two sensing regions. Our goal is to highlight elastin 2PF against cellular 2PF (NADH). This added contrast will help clinicians identify stromal boundaries even in severely sun damaged skin. We will test this sensing architecture on our automated slide scanning system. Our clinical readers will provide a head-to-head comparison of the two sensing arrangements on identical samples.

We have instituted a four-phase stage-gate design control process through our standard operating procedure to advance our technology to a commercially viable state (Fig. C1). These phases are: Planning & Design Input (Phase 1), Verification and Validation Readiness (Phase 2), Verification and Validation Completion (Phase 3), and Design Transfer (Phase 4). Each phase is completed via a formal design review where we consider our regulatory plan, regulatory requirements, clinical study plans, usability engineering process, and resource needs. We have also instituted an independent software development plan in accordance with the primary standard IEC 62304. Between Phases 1 and 2, we will conduct an intermediate Clinical Readiness Design Review where a subset of the Phase 2 requirements will be completed to permit safe initiation of our clinical investigations (both for 510 (k) approval and our Biopsy Equivalence Study, see Commercialization Plan).

These design reviews ensure that we build the correct device for clinician needs and build the device correctly and reproducibly. A major activity in Phase 1 is to characterize and document user needs for our product. We have already collected valuable feedback from real-world clinicians, and we will continue to do so through the remainder of our Phase II SBIR. Other important outputs of our Phase 1 design review are completion of our requirement specification, layout of the overall product development plan, use risk analysis, system and hardware risk analysis, GUI risk analysis, firmware risk analysis, and the release of our quality plan along with associated standards for product hardware, software, labeling, regulatory considerations and our quality system requirements. We will complete requirements for our Phase 2 design review that ensure a level of performance and safety sufficient for conducting a clinical trial. These include design inputs, software requirement specification, design verification and validation plan, and our device master record.

Aim 1 Risks: Our design process in Aim 1 will minimize the risk of building a device that is unsafe, non-reproduceable, or unable to satisfy the needs of our users. Identifying or implementing design changes after we have committed to a design plan is a possible risk that could extend timelines and development expenses. We will mitigate the risk of late changes by utilizing the remainder of our Phase II project and the early part of this Phase IIB project to test our design with more users and more live subjects.

Aim 1 Milestones: Successfully implement design modifications for rapid ambient light mitigation and spectral splitting to both satisfy human factors requirements (100% of 15 users make no critical use mistakes in second round of testing) and to prepare for scaled commercialization. Advance our device and documentation to a level of Clinical Verification and Validation Readiness and complete formal Clinical Readiness Design Review.

Phase IIB Aim 2 | Conduct trial to support reimbursement body of literature (24 months).

Aim 2 Rationale: Reimbursement by payers and clinical adoption of this novel MPM skin-imaging technology will proceed in a stepwise fashion to limit risk to patients and build a convincing body of real-world clinical evidence to support more diagnostic marketing claims. The broad study we describe here represents an essential and substantial pier in this body of evidence.

Additional studies required for widespread reimbursement and clinical adoption will be conducted with third party funding. These additional studies include smaller but targeted investigations of use cases with highest unmet clinical need but low incidence for early commercialization (see Commercialization Plan).

Feedback from dermatology clinicians and reimbursement experts has emphasized the need for a convincing determination of diagnostic sensitivity and specificity. By the end of Phase IIB Aim 2, we will have gathered the in vivo validation data required for a high quality peer-reviewed publication that will provide statistical support for a positive payment decision. This broad statistically-powered study, paired with stepwise investigations of targeted use-cases, will advance commercialization of this technology to the patients most in need.

Aim 2 Methods: We will prospectively recruit 300 patients at six dermatology clinic sites to assess the performance of physician diagnosis of suspected BCC when using the EnSpectra device and clinical observation. We will power the study ($1-\beta = 0.9$, target $\alpha = 0.05$) to reject the null hypothesis of 80% sensitivity, based on an expected true rate of 90% conservatively estimated from Phase II Aim 2 sensitivity

results (94.7% sensitivity). We estimate a study with 150 BCC-positive patients will achieve significance, with a total of 300 patients assuming 50% of recruited patients have BCC. Specificity of BCC diagnosis, interrater agreement, and management decision (biopsy vs reassure) sensitivity and specificity will be secondary endpoints. Clinical trial investigators will collect the following data: (1) EnSpectra MPM images of equivocal lesions, (2) EnSpectra MPM images of nearby or contralateral uninvolved skin, if possible, (3) dermatoscopic and macroscopic images of skin condition, (4) patient history and demographics, (5) full differential before biopsy results, (6) pathology slides, if taken (reader blinded), and (7) ultimate diagnosis (reader blinded).

A panel of 5 expert readers, blinded to pathology slide images and the ultimate diagnosis, will receive information for each sample (Items 1 – 5, above), and provide: (a) a primary diagnosis, (b) an estimate of diagnosis confidence, and (c) a management recommendation (biopsy lesion or reassure patient).

We will also demonstrate device safety. Because of the safety profile of the device, we do not expect to observe any serious adverse events during the clinical trial. Adverse events with a causal relationship with device use are expected to be very rare, if they occur at all. We expect the overall adverse event rate to be less than 2%.

Aim 2 Risks: The primary risk in conducting a clinical trial in the near future is the ongoing impact of Covid-19 that could slow patient recruitment; many NMSC patients seem to be avoiding visiting dermatologists[28] presumably to avoid infection. To reduce this risk, we have delayed the onset of this important trial to complete additional technological innovation that will improve device performance in Aim 1. Should patients continue to avoid dermatologic care beyond this time, recruitment for the trial may be slow, and we will consider a combination of the following actions: open additional, parallel clinical trial sites and/or extend the duration of the trial to account for slower recruitment using third-party funds.

Aim 2 Milestones: By the end of Phase IIB Aim 2, we will have gathered the in vivo validation data that validates the primary BCC sensitivity endpoint required for a high quality peer-reviewed publication that will provide support for a positive payment decision.

COMMERCIALIZATION READINESS AND COMPETITIVE ADVANTAGE

Our mission is to transform disease diagnosis by enabling noninvasive, point-of-care, digital histopathology in living epithelia through clinical multiphoton microscopy. Creating a paradigm shift of this magnitude necessitates a stepwise approach, beginning with a beachhead opportunity from an important unmet clinical and economic need. In this proposal, we describe such an opportunity: noninvasive diagnosis of nonmelanoma skin cancer (NMSC). We validated the importance of this need through real-world observations of clinical practice and dozens of patient and clinician interviews. We further quantified estimated market penetration and revenue acceleration using Center for Medicare and Medicaid Services (CMS) databases and peer-reviewed scientific literature. Our plan for evidence development that will lead to clinical adoption and reimbursement of our technology stems from extensive clinician feedback and the reactions of care providers, medical societies, and insurance providers. We crafted our commercial strategy with careful consideration for our financing requirements and the need to appeal to third party investors and potential corporate partners.

Our solution is a digitally enabled, diagnostic platform that puts the power of immediate, noninvasive histopathology in the hands of clinicians. Our commercial strategy places EnSpectra at the center of this platform, facilitating the immediate gathering, sharing, processing, and interpreting of skin cellular imaging data by dermatologists and pathologists. Our platform will substantially benefit patients, clinicians, and payers by accelerating diagnoses and enabling new opportunities to identify and treat skin cancers earlier. In our Commercialization Plan, we outline a strategy to initially follow a predicate technology through the FDA 510(k) regulatory process with a screening indication, followed by a diagnostic claim for non-inferiority to traditional biopsy. Additionally, we provide a description of our product launch process (Fig. C1). These strategies create an opportunity for earlier revenue, reduced capital needs, and shorter time to market.

EnSpectra Health is uniquely positioned to address this longstanding need in dermatologic oncology. We have a unique technologic advantage and have proven our ability to execute our innovation through the lifetime of our Phase I and Phase II projects (see Progress Report). Our innovations span multiple disciplines, and in many cases are the first of their kind. As described in our Commercialization Plan, these innovations create a dual barrier to our competition of robust intellectual property and in-house skill and knowledge. Furthermore, our ability to successfully fundraise has accelerated our pathway towards commercialization. EnSpectra is ahead of the competition, and we are seeking this Phase IIB award to accelerate further.

MILESTONES | For Aim Milestones, see APPROACH; for summary, see Commercialization Plan (Fig. C1).

Patents:

PCT/US2018/030011

PCT/US2019/061306

US Provisional Patent Application: 63/023,727

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 02/28/2023

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data * ● Yes ❍ No

Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.

Are Human Subjects Involved ● Yes ❍ No

Is the Project Exempt from Federal regulations? ❍ Yes ● No

Exemption Number ❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8

Other Requested Information

Human Subject Studies

Study#	Study Title	Clinical Trial?
The form does not have any study records		

Delayed Onset Studies

Delayed Onset Study#	Study Title	Anticipated Clinical Trial?	Justification
1	Biopsy Equivalency Study	Yes	CLINICALTRIALJUSTIFICATION.pdf

Justification for Delayed Onset Study

Human subjects study information is not available at this time because we are developing the trial design and protocol with the assistance of our clinician consultants, clinical/regulatory consultant and biostatistician (see support letters), and also expect additional trial design input to be generated from trials to be conducted in the coming year. As the purpose of this study is to generate data for reimbursement for commercialization, this effort is critical to the success of our company. In the subsequent paragraphs we outline our plans to protect subjects and ensure appropriate inclusiveness in our recruitment and have included our current procedures for conducting and monitoring clinical studies.

Protection of Human Subjects

The sections below describe the need for human subjects, special populations, measures taken to protect study participants, and considerations related to the use of children and the inclusion of both genders and all ethnic/racial categories as human subjects.

Risks to Human Subjects

Human Subjects Involvement, Characteristics and Design

We require the participation of human subjects as it is the end goal for our technology to be useful on live human subjects. Our device is a nonsignificant risk device (NSR), and given the low risk it poses to humans, it is more appropriate to proceed with imaging on human skin rather than animals. This simplifies our experimental approach as we do not need facilities and protocols for animals. Imaging live human skin directly further eliminates potential differences that may exist between animal skin and/or chemically fixed or treated human skin that may confound our data.

Our subjects will be recruited at six (6) dermatology clinical trial sites. Trained users will image patients that will either receive a biopsy for a suspected disease or receive a clinical diagnosis. Patients with various skin diseases may be included, but the majority of biopsies required in dermatology clinics are for suspected nonmelanoma skin cancer. Based on the demographics of nonmelanoma skin cancers, the subjects are most likely to be Caucasian with a slight bias towards male subjects (55%) versus female subjects (45%).

We will allow for the recruitment of children because the use of a noninvasive alternative to biopsy may be particularly advantageous in pediatric populations. We will not, however, specifically target enrollment of children or require a certain percentage of patients to be children. We will not involve any other special vulnerable populations.

There will be no assignment of patients to study groups.

EnSpectra will control all data collection and management. Subject data will be maintained on an encrypted external hard drive that will remain in a locked location or an encrypted HIPAA-compliant cloud service. We will conform with all HIPAA requirements as outlined in our training for IRB approval (expected to be Salus IRB).

Sources of Material

We will record digital images of the patients' skin disease using our noninvasive imaging probe. We will also record images of uninvolved skin to serve as an internal control for comparison. We will record subject data such as age, diagnosis, and location. Subject data will be maintained on an encrypted external hard drive that will remain in a locked location or an encrypted HIPAA-compliant cloud service. We will conform with all HIPAA requirements as outlined in our training for IRB approval.

Potential Risks

The potential risk to subjects in this study is very low as our system is a nonsignificant risk device (NSR) as determined by the FDA concurrence of our predicate device. Our skin imaging device is under a currently active study within EnSpectra via private IRB approval (Salus IRB). A skin-imaging study of healthy skin within EnSpectra has been considered to be 'no more than minimal risk' by Salus IRB.

There is no medical benefit associated with this imaging within the proposed study, as it will not affect treatment of subjects, render a diagnosis, or provide information to their dermatologist regarding our data. We will have independent dermatologists assess our images and will observe whether they render the same diagnosis as the primary care provider. Having conducted investigator sponsored human studies within EnSpectra under IRB oversight, we have the necessary training, protocols, and hazard assessment controls in place to assure safe treatment of our subjects.

Adequacy of Protection Against Risks

Recruitment and Informed Consent

Potential subjects will be screened for inclusion criteria verbally and by observation of the investigators. In the consent form, we will ask if the subject is participating in other protocols. We will also verbally ask the potential participant this question. A few hours will be enough time to prepare the setup, consent the subject and conduct the imaging protocol. Analysis of participant image data will take several days. Subjects will be provided a written consent form, outlining the purpose, risks, and process of the experiment as well as precautions for protection of identifying information.

Prior to providing informed consent regarding participation, patients will be provided a copy of the study protocol and consent form and will be encouraged to discuss potential risks and benefits of study participation with the Principal Investigator or another qualified Investigator. Consent will be documented in compliance with IRB guidelines. We will monitor the age and ethnic, racial and gender characteristics of all patients enrolled. Given the general populations that receive skin biopsies, represented predominantly by patients with suspected nonmelanoma skin cancer, we will aim to recruit at least 10% of subjects with African, Asian, Hispanic, or any non-Caucasian ancestry. All subjects enrolled in this study must sign an informed consent prior to the imaging session. A copy of this form is given to the subject. Consent forms and approval documentation and related correspondence will be provided upon request.

Protections Against Risk

We will make every attempt to minimize or eliminate potential risks to research subjects. To protect the eyes from laser exposure, laser light will be contained by the components of the system, and other appropriate risk mitigation procedures will be in place.

We have conducted a thorough hazard and risk analysis as a process of our quality control and IRB application. We will have an independent expert physician serve as a designated medical monitor to assess and document any adverse events. A clinical trial expert will serve as an independent monitor of the study data.

Besides contact information (name, date, telephone number and email address), no individually identifiable information will be collected. A subject may choose to terminate her/his participation at any point in the study. A subject's involvement will be complete after we collect images as described in this application. Basic first aid materials will be on hand. The data collected, namely images of skin structures within the subjects without any accompanying identifying text, will not represent PHI as there is no means to identify an individual with any specific image at that scale.

This study does not involve ongoing interventions. Study data collected consists of images of skin cellular structures. If any adverse events or unanticipated problems (UPs) occur during an imaging session, those will be recorded. UPs will be recorded and reported (within 10 working days via the IRB Report Form). Any adverse event or UP that occurs after the imaging session will be documented upon researchers finding out about the event, and UPs will be reported directly to the IRB within 10 working days (or according to IRB guidance). As part of the consent process, subjects are instructed to contact the protocol directors to report any adverse events that occur within 1 week of participation in the study.

Potential Benefits of the Proposed Research to Human Subjects and Others

There are no associated health benefits with this procedure for the patient. The benefits derived from these studies are for validation of the technology only.

Importance of the knowledge gained

The value of these experiments will be to demonstrate the ability for rapid, minimally invasive investigation of living skin structure. These experiments will validate that our technology images with sufficient resolution and quality to identify features of skin. There is a tremendous scientific and medical benefit to this type of real time pathology. Given that our skin imaging system is completely noninvasive, we are confident that any procedure risks are outweighed by the vast medical and scientific benefits that may be realized.

Inclusion of Women and Minorities

Our proposed experiments offer no medical benefits and are purely for the purposes of testing and validating our device. We will not discriminate our recruitment based on gender or ethnicity.

We have set enrollment targets for our study group that match on a proportional basis the composition of the US population that are affected by NMSC. NMSC predominantly affects people that are White (>80%), followed by Hispanic or Latino and Asian. We will aim to recruit at least 10% of subjects with Hispanic or Latino, Asian, African, or any non-

Caucasian ancestry. Although men are more likely to develop NMSC, we will make our best effort to recruit approximately 50% women and 50% men in our clinical trial.

In collecting data on ethnicity, we plan to use a standard form with a field on which the subject may select an ethnic category (Hispanic or Latino vs. non-Hispanic/Latino) and, following this field a second field on which the subject may select one or more racial category. Reporting on Gender, Ethnicity and Racial categories will follow Federal Guidelines and will include an enumeration of subjects reporting more than one racial category. We will make our best effort to mirror the demographic breakdown outlined above.

Although skin cancer predominantly affects people with lighter skin, we are committed to optimizing and testing our device on diverse colors of skin so that people of color with skin cancer will not be denied access to the improved care offered by our technology, especially since skin pigmentation may have an effect on imaging.

Clinical Operating Procedure (attached below is our procedure for Clinical Study Conduct)

1. PURPOSE

The EnSpectra Health Clinical department prepares and conducts clinical studies designed to assess the safety and/or performance of medical devices for regulatory purposes. This procedure provides an overview of the clinical study process, including design, obtaining regulatory approval, conduct, recording and reporting data, and closeout. The main objectives of the procedures in this and related procedures are to protect human subjects, maintain the integrity of study data, and ensure compliance with applicable regulations.

2. SCOPE

This procedure applies to clinical investigations carried out in human subjects to assess the safety and/or performance of medical products for regulatory purposes when EnSpectra Health serves as the Sponsor.

3. RESPONSIBILITIES

Executive Management	• Make final decision to conduct a clinical study and provide resources necessary for studies.
Clinical Management	• Advise Executive Management regarding when a clinical study may be necessary.
	• Ensure personnel are adequately qualified, providing training on how to conduct clinical research responsibilities, supervising the conduct of clinical studies, and overseeing any third parties who have been delegated duties in the clinical study
	• Assume responsibility of all aspects of the following: conducting the study, including planning and designing the study; preparing the required clinical deliverables; working with Regulatory Affairs to submit applications to regulatory authorities; conducting, monitoring and closing the study; analyzing data; creating reports; archiving study data and ensuring all reporting obligations are fulfilled.
All parties	• Share the responsibility for the ethical conduct of the study in accordance with their respective roles in the study.

4. RECORDS

There are no records associated with this procedure.

5. FORMS & APPENDICES

[redacted] Acronyms & Definitions

6. RESOURCES

6.1. US FDA Regulations, including

6.1.1. Protection of Human Subjects – 21 CFR 50

6.1.2. Financial Disclosure by Clinical Investigators – 21 CFR 54

6.1.3. Institutional Review Boards – 21 CFR 56

6.1.4. Investigational Device Exemptions – 21 CFR 812

6.2. European Commission Medical Device Directive 93/42/EEC, as amended

6.3. ISO 14155 – Clinical investigation of medical devices for human subjects — Good clinical practice

6.4. ISO 14971 – Medical Devices – Application of risk management to medical devices

6.5. Additional regulations may be applicable depending on the location of the study; these will be specified in the clinical study protocol.

7. REVISION HISTORY

Rev #	Date	Description	CO No.
0	2016-06-13	Initial release	10005
1	2018-04-04	Added new form [redacted]	10021
2	TBD	Release into EnSpectra Health QMS. Minor updates associated with transferring into new system, new company name.	P0018

8. CLINICAL STUDY PROCESS OVERVIEW

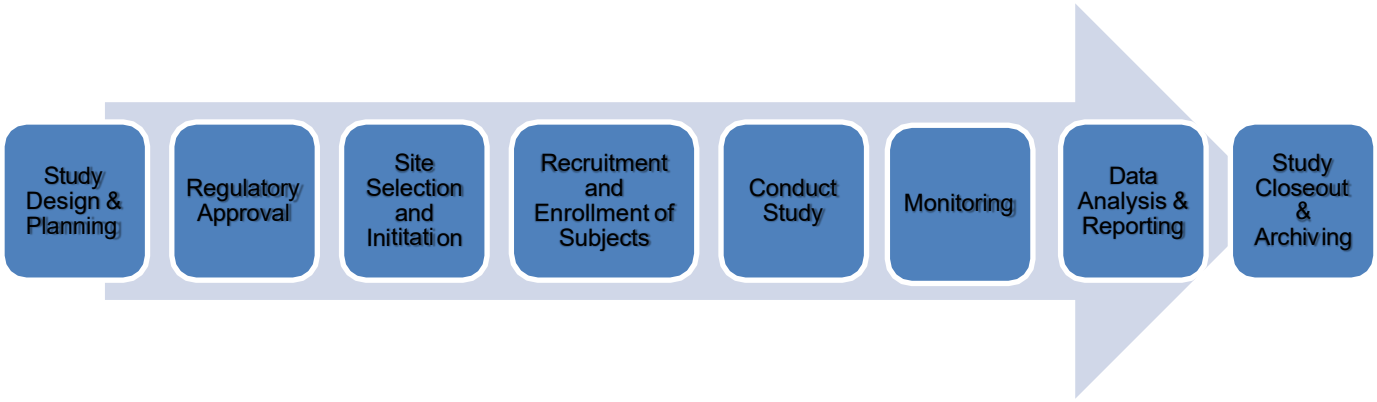


Figure 1. Overview of the clinical study process. Some steps may happen in a different order or concurrently with other steps.

9. PROCEDURE

9.1. Study design and planning

9.1.2. Once the decision to conduct a study has been made, the EnSpectra Health Clinical department plans and designs the study. The clinical study should be designed to evaluate whether the investigational device or procedure is suitable for the purpose(s) and the population(s) for which it is intended, ensuring that the results have clinical relevance and scientific validity.

9.1.3. A project budget shall be created.

9.1.4. A Trial Master File shall be created and maintained. See [redacted] Sponsor-Study Documentation Guidelines.

9.1.5. A risk analysis shall be conducted. See [redacted] Sponsor-Study Documentation Guidelines.

9.1.6. A study protocol shall be drafted. See [redacted] Sponsor-Study Documentation Guidelines.

9.1.7. The following documents may be created for the study, or, the related information may also be captured in the study protocol in lieu of separate documents.

- *An Investigator's Brochure (IB) as necessary. See [redacted] Sponsor-Study Documentation Guidelines.*
- *Monitoring Plan. See COP-02 Sponsor-Study Documentation Guidelines.*

- *Labeling*

9.1.8. A Data Monitoring Committee may be established, but is not required, in order to enhance the safety of study participants (for example, in situations in which safety concerns may be unusually high). An Endpoint Assessment/Adjudication Committee may be established to review study events or endpoints (for example, when the intervention is not delivered in a blinded fashion). If a committee is established, their function should be delineated in the study protocol.

9.1.9. If any of the EnSpectra Health Clinical functions are outsourced to vendors, Sponsor must specify in writing which duties or functions are assumed by the external organization.

9.1.10. Audits may be conducted for quality assurance of study conduct or monitoring. Audits should be conducted if there are repeated major study protocol deviations, suspicion of fraud, or at the request of a regulatory agency.

9.2. Regulatory approval

9.2.1. EnSpectra Health Clinical shall require written approval or waiver from the Institutional Review Board or Ethics Committee before it notifies a study site to begin the study.

9.2.2. EnSpectra Health Clinical will determine if other regulatory approvals are required. If required, written approval from a National/Competent Authority must be documented before EnSpectra Health Clinical notifies a study site to begin the study.

9.2.3. Amendments to the study protocol and other reviewed documents should be reviewed and approved by the original reviewing body, unless they are non-substantial changes which do not affect the rights, safety and well-being of human subjects or are not related to the clinical study or endpoints. Non-substantial changes may be sent as a notification or filed in the study TMF as provided by the governing body's guidelines.

9.3. Site selection and study set-up

9.3.1. Site and Investigator Selection

- *Study investigators selected to participate must have adequate qualifications, adequate resources, and access to adequate number of subjects.*
- *The names, initials, signatures, functions and designated authorizations of the study personnel must be documented.*

9.3.2. Investigators and Site Personnel must be adequately trained; and this training must be documented. New Investigators and Site Personnel may be added during the trial if they are sufficiently trained.

9.3.3. An Investigator Agreement must be signed between Sponsor and the Investigators to delineate each party's responsibility. The agreement shall indicate that the Investigators may share some regulatory responsibilities with the Sponsor by participating in a clinical study.

- *For Sponsor in-house run studies, a consulting or employment contract may be used in lieu of an Investigator Agreement.*

9.3.4. Investigator selection must be carried out to minimize bias, especially in regards to the financial interest of the Investigator in the study outcome. Prior to study start, the Investigator must provide information regarding the Investigator's financial relationship with Sponsor.

9.4. Recruitment, enrollment, and protection of subjects

9.4.1. Subject payments should fairly compensate the subjects for their time and expenses related to the study and should not constitute improper inducement that unduly encourages the subject to participate.

9.4.2. Subjects who suffer an adverse event as a result of participating in the clinical study should be provided additional health care.

9.4.3. Informed Consent must be obtained in writing from the subject before any procedure specific to the clinical study is applied to the subject, including screening or data gathering.

9.4.4. The Investigator will maintain a log to record subject identification to ensure that the case report forms can be reconciled with the source records. The presence of the log will be verified during the site initiation visit.

9.4.5. All subjects who enroll in the study must be accounted for and ultimate disposition documented including the reason for withdrawal, if relevant.

9.5. Study conduct

9.5.1. The study must be conducted in accordance with the study protocol.

- *If there is any change, divergence, or departure from the study design or procedures of a research protocol that is under the Investigator's control and that has not been approved by the governing Institutional Review Board or Ethics Committee, a deviation must be recorded.*
- *A minor protocol deviation is a change or alteration in the conduct of the trial which does not have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy and reliability of the study data. Even if a single or infrequent occurrence may be considered a minor deviation, if it is discovered these events have involved a majority of research subjects or the frequency is increasing, this may signify a more systemic problem with the conduct of the research and this could lead to reclassification of the events as major protocol deviations.*
- *A major protocol deviation is a change or alteration in the conduct of the trial which that may affect the subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data.*

9.5.2. If an Investigational Device is used, Sponsor must ensure device accountability.

9.5.3. Adverse events must be recorded and reported.

9.5.4. Sponsor may suspend or prematurely terminate the study or the participation of an individual site for significant and documented reasons such as a suspicion of unacceptable risk to subjects or major or repeated deviations by a study investigator. The decision to terminate the study or a site must be justified in writing and communicated to the relevant Ethics Committee, Institutional Review Board and/or Competent Authority.

- *If the study will resume after a temporary suspension, Sponsor must notify the principal investigators, Ethics Committees and Institutional Review Boards, and relevant Competent Authority if required by regulations.*
- *If a site will resume after a temporary suspension, Sponsor must notify the Ethics Committees or Institutional Review Board and relevant Competent Authority if required by regulations.*

9.6. Study monitoring

9.6.1. The main purpose of Study Monitoring is to verify that the conduct of the clinical study complies with the approved study protocol, subsequent amendment(s), ISO 14155, Good Clinical Practices and all other applicable regulatory requirements.

9.7. Event reporting

9.7.1. Adverse event reporting shall be conducted.

9.7.2. The following must be reported to the Institutional Review Board or Ethics Committee:

- *Major deviations*
- *Regular progress reporting, including safety summaries and reports of minor deviations*
- *Withdrawal of competent authority approval*
- *Notification of study suspension or premature termination*
- *Recall or field safety correction of an investigational device*
- *Completion or termination of a significant risk device investigation*
- *Final study report*

9.7.3. The following must be reported to US FDA if the study is conducted under an IDE:

- *Regular progress reporting, including safety summaries and reports of minor deviations*
- *Current list of the names and addresses of all Investigators participating in a significant risk device investigation (every six months)*
- *Institutional Review Board determination that an investigational device is a significant risk device and not a non-significant risk device as the sponsor had proposed to the Institutional Review Board*
- *Notification of study suspension or premature termination*
- *Recall or field safety correction of an investigational device*
- *Any Investigator use of an investigational device without first obtaining informed consent*
- *Completion or termination of a significant risk device investigation*

- *Final study report, which should include a certification or information regarding financial arrangements with the Investigators described in 21 CFR 54.*

9.7.4. The following must be reported to study Investigators:

- *Withdrawal of Competent Authority approval*
- *Institutional Review Board or Ethics Committee approval withdrawal*

9.8. Data analysis

9.8.1. Data analysis shall be conducted in accordance with each study's protocol.

9.9. Study close-out and archiving

9.9.1. The purpose of study close-out is to ensure that all investigator records are complete, all documents needed for Sponsor's trial master file are retrieved, remaining clinical investigation materials are disposed of, previously identified issues have been resolved and all parties are notified of study close-out.

9.9.2. After close-out of the clinical investigation, a written report of the clinical evaluation shall be completed in accordance with the applicable regulations, even if the clinical investigation was terminated prematurely.

9.9.3. Sponsor and study investigators shall maintain the clinical investigation documents as required by the applicable regulatory requirement(s). They shall take measures to prevent accidental or premature destruction of these documents. Custody of records may be transferred to another person/party as long as the transfer is documented at the investigation site or at the sponsor's facility.

Data and Safety Monitoring Plan (attached below is our procedure for Clinical Study Monitoring)

1. PURPOSE

This procedure defines the processes for EnSpectra Health Clinical when conducting clinical study monitoring.

2. SCOPE

- This procedure applies to all clinical studies of an Investigational Device that are EnSpectra-initiated.
- This procedure applies to all monitoring conducted for a study, including in-person monitoring visits, centralized and remote monitoring.
- This procedure does not apply to investigator-initiated studies sponsored by EnSpectra or in-house Sponsor-Investigator studies, although this procedure may be used as a guideline.

3. RESPONSIBILITIES

EnSpectra Clinical	• Oversee implementation and maintenance of this procedure.
Clinical Monitors	• Carry out the activities noted in this procedure.

4. RECORDS

There are no records associated with this procedure.

5. FORMS & APPENDICES

[redacted] Acronyms & Definitions

6. REVISION HISTORY

Rev #	Date	Description	CO No.
0	2016-06-13	Initial release	10005

7. PROCEDURE

Except where otherwise stated, these activities need not be carried out in the order listed.

7.1. Monitoring Plan

7.1.1. A monitoring plan should be created to describe the monitoring methods, responsibilities, and requirements for the study.

7.1.2. The monitoring plan should include the following:

- *A brief description of the study, its objectives, and the critical data and study procedures, with particular attention to data and procedures that are unusual and require on-site training*
- *Description of each monitoring method (e.g., On-site, remote, centralized) to be employed during the study and how it will be used to address important risks and ensure the validity of critical data*
- *Timing, frequency, and intensity of planned monitoring activities*
- *Specific activities or documentation required for each monitoring method employed during the study, including reference to required tools, logs, or templates. If different from templates, format, content, timing, and archiving requirements for reports and other documentation of monitoring activities should be specified.*
- *Process for appropriate communication of routine monitoring results to management and other stakeholders (e.g., CRO and data management); of immediate reporting of significant monitoring issues to appropriate personnel, and from study management and other stakeholders to monitors*
- *Process for addressing unresolved or significant issues (e.g., significant non-compliance with the investigational plan) identified by monitoring, whether at a particular site or across study sites*
- *Other aspects that may (optionally) be addressed in the monitoring plan include:*
    - *Definitions of events or results that trigger changes in planned monitoring activities for a particular clinical investigator. For example, if it is determined that an investigator deviates significantly from other sites in making safety-related findings or other key safety metrics, the site may be considered for targeted on-site visits. Additional examples of potential triggers include suspected fraud, data outliers (e.g., in rate of enrollment, volume of protocol deviations, or quantity of adverse event/effect reporting), or delays in completing CRFs.*
    - *Plan for monitoring of quality to ensure that sponsor and CRO staff conduct monitoring activities in accordance with the monitoring plan, applicable regulations, guidance, and sponsor policies, procedures, templates, and other study plans*
    - *Other quality management practices applicable to the clinical investigation (e.g., reference to any other written documents describing appropriate actions regarding non-compliance)*
    - *Description of any specific training required for personnel carrying out monitoring activities, including personnel conducting internal data monitoring, statistical monitoring, or other centralized review activities*
    - *Processes to ensure that root cause analyses are conducted where important deviations are discovered and that appropriate corrective and preventive actions are implemented to address issues identified by monitoring.*

7.1.3. The monitoring plan should be reviewed periodically during the course of the study, and revised if needed to ensure inclusion of additional information or procedures that become apparent during the conduct of the study.

7.2. Remote monitoring

7.2.1. Remote monitoring of any copies of Trial Master File data that can be accessed away from the study site may occur. (e.g., source worksheets, regulatory approvals)

7.2.2. If both source records (including worksheets) and Case Report Forms may be accessed away from the study site, remote monitoring of Case Report Forms may be performed.

7.2.3. If copies of source records are sent to Sponsor, these source records should not contain patient-identifiable data. If source record copies with patient-identifiable data are sent to Sponsor, the site should be notified to redact records before sending them to Sponsor. Any copies in Sponsor' possession should be redacted. This guideline is not applicable for in-house, Sponsor-Investigator studies.

7.2.4. Source records should be compared against the Case Report Forms to verify that the data are consistent.

- *The data points to be monitored may be defined by the monitoring plan, study protocol, or by other documentation. If not defined, the following should be monitored at a minimum: eligibility criteria, data*

related to study endpoints (both primary and secondary), adverse events, protocol-required safety assessments.

- *If data are inconsistent with or not supported with the source documents, the Monitor should generate a query.*

7.2.5. Source records should be checked for adherence to investigator agreement, study protocol, and any other study requirements.

7.2.6. If an Electronic Data Capture system is used, automatic queries are generated when the data entered on an electronic Case Report Form violates the expected range of values for that data (edit check).

7.2.7. For both manual and automatic queries, the site research coordinator or investigator responds to the query by changing the data item or reconfirming the data's accuracy. A reason must be given for either the change or leaving the original data the same.

7.2.8. An audit trail must be created each time data are entered or modified. The audit trail will include a record of changes made to the data value of the item, the reason for the change, the date the change was made and the identity of the person making the change.

7.3. Centralized monitoring

7.3.1. If appropriate to the study, centralized monitoring may occur. The following are examples of centralized monitoring: statistical analyses to identify data trends, review of unusual distribution of data (such as too little variance), review of inconsistent data or data outliers, review of eCRF data to uncover potential protocol deviations.

7.3.2. If used, centralized monitoring may guide on-site monitoring. For example, reviewing analysis or listing of performance metrics, noncompliance, or rates of repeated deviations may be useful when determining the on-site monitoring frequency and focus for a particular site.

7.4. Site qualification

7.4.1. The Monitor shall assess the site to verify that the investigator has adequate qualifications, adequate resources and access to an adequate number of subjects.

7.4.2. All personnel involved in clinical study conduct should be qualified by training and experience to perform their tasks.

7.4.3. Site qualification may be done by telephone if the study site has participated in a previous Sponsor-sponsored study or if Sponsor personnel are familiar with the investigator's qualifications, resources, and access to subjects. Site qualification may also be combined with Site Initiation.

7.4.4. The rationale for selection of the study site must be documented. COP-01-03 Study Site Qualification Report Form may be used; a memo or other means of documentation will also suffice.

7.4.5. For Sponsor in-house studies, documentation of study site suitability may be made in the IRB application.

7.5. Site initiation

7.5.1. Either a Site Initiation Visit or an Investigator Meeting should be conducted prior to study start of each site.

- *For Sponsor in-house studies, site initiation may be waived.*

7.5.2. The monitor shall confirm that:

- *All regulatory approvals have been obtained. See COP-01-06 Study Site Regulatory Binder Checklist.*
- *The investigator is familiar with and accepts his or her obligations to conduct the study in accordance with applicable regulations and Good Clinical Practices.*
- *The Investigator has access to the required devices and/or Sponsor-provided resources.*
- *The site personnel have received and understood the requirements of all study documents.*
- *The site personnel have been trained in investigational procedures and devices, study conduct, and the Electronic Data Capture system (if applicable) and are familiar with their responsibilities.*
- *An investigator meeting may be conducted in lieu of the on-site initiation visit.*
- *Following the site initiation visit, the monitor should complete COP-01-04 Study Site Initiation Report Form, or equivalent.*
- *A follow-up communication should also be sent to the site, if appropriate.*

7.6. On-site interim monitoring

7.6.1. Prior to the monitoring visit, the Monitor will:

- *Review the Trial Master File to check if there is any incomplete/ missing site documentation that needs to be resolved at the monitoring visit.*
- *Check whether staff or organizational changes would impact the validity of the Clinical Study agreements or necessitate revision of the signature page on the protocol and/or COP-01-05 Study Delegation of Authority Signature Log.*
- *Review current status of study, including patient enrollment rates and patient progress; reported Serious Adverse Events; and inventory of investigational devices sent to site, if applicable.*
- *Review last monitoring report and recent communications and note whether outstanding actions and requests need to be addressed at the visit.*
- *Schedule a visit with investigator and study coordinator noting time, date, venue, expected duration of visit and outlining the purpose of the visit, items for discussion and state if any other personnel need to be present.*
- *Send confirmation letter, email or fax to investigator, giving details of the scheduled visit and informing the investigator of documentation/ information that must be made available at the meeting.*

7.6.2. The Monitor will review some or all of the following areas during the monitoring visit:

- *Check adherence to protocol, investigator agreement, and other applicable documentation.*
- *Ask investigators whether protocol deviations have occurred (e.g. adherence to time intervals, blinding, recording of Adverse Events, etc.). Protocol deviations may also become evident during the source document verification.*
- *Ask investigators whether there have been any recent changes (or if changes are imminent) in the site personnel and confirm that the COP-01-05 Study Delegation of Authority Signature Log has been amended appropriately. Confirm that additional personnel have been appropriately trained.*
- *The Investigator's Site Regulatory Binder should be reviewed for completeness. See COP-01-06 Study Site Regulatory Binder Checklist. If any documents are missing and are unable to be obtained during the monitoring visit, an observation should be made on the visit report.*
- *If an investigational device is utilized for the trial, check investigational device supplies. Confirm inventory; ensure that storage requirements are met; review accountability procedures; review ordering requirements against current supply.*
- *Check adherence to informed consent process. Verify appropriate written informed consent has been obtained for subjects. Check that the date of informed consent shows that informed consent was obtained before any study-specific procedures or screening was performed.*
- *In the event that an investigational device is used on a patient who has not signed an informed consent, notification must be made to both FDA if the study is conducted under an IDE and the reviewing Institutional Review Board/Ethics Committee within 5 working days of discovering the issue.*
- *Check whether any adverse events have occurred since the last monitoring visit. Ensure that all adverse events have been recorded and reported correctly.*

7.6.3. Following the monitoring visit:

- *Complete* [redacted] *Study Site Interim Report Form or equivalent.*
- *Provide follow-up information and make additional documentation requests as necessary.*
- *A follow-up communication should be sent to the site, if appropriate.*
- *File the monitoring report and follow-up letter in the Trial Master File.*

7.7. Close-out visit

7.7.1. The following should be completed during the close-out visit.

- *Confirm CRFs complete.*
- *Confirm outstanding queries and deviations are resolved.*

- *Confirm that all adverse events are documented.*
- *Confirm that Site Regulatory Binder (or electronic equivalent) is complete.*
- *Ensure disposition of investigational devices, remaining samples and other clinical investigation materials is completed and documented.*
- *Ensure that the ethics committee or institutional review board is notified of study closure.*
- *Ensure that all records are appropriately archived and retained.*
- *Update the investigator's information regarding financial relationship with Sponsor. See [redacted] Investigator Financial Disclosure Form.*

7.7.2. Complete [redacted] Study Site Final Monitoring Visit Report Form.

7.7.3. If there are any actions items from the Close-out Visit, a [redacted] Close-Out Memo-to-File should be completed to show action item completion.

7.7.4. A follow-up communication should also be sent to the site, if appropriate.

7.8. Documentation of visit

7.8.1. Each monitoring visit should be documented by the Monitor signing the [redacted] Study Site Visit Log.

7.9. Addressing deviations

7.9.1. Deviations from the signed Investigator Agreement, study protocol, IDE regulations, or any conditions of approval imposed by the FDA or other health agency or reviewing Institutional Review Board, are to be reported on the [redacted] Deviation Form. The Deviation Form should be completed by the Monitor or designee and a copy filed with the associated material in the Site Regulatory Binder at the investigational site as well as a copy filed in the Trial Master File.

7.9.2. Upon discovery of a protocol deviation and depending on the nature of the deviation, Sponsor must secure compliance and require the investigator to take corrective measures or discontinue shipments of the device to the investigator. Repetitive and/or significant deviations from the study protocol may also lead to termination of the Investigator's participation in the study.

- *If the Investigator's participation in the study is terminated, the Investigator must return or dispose of the unused devices as authorized by Sponsor, unless this action would jeopardize the rights, safety or welfare of a subject. Termination of an investigator must be reported to the FDA (if U.S. site) and Institutional Review Board/EC.*
- *If a terminated investigator's participation in the study is to be resumed, approval from the FDA (if U.S. site) and Institutional Review Board/Ethics Committee must be received prior to resuming the investigator's participation.*

7.9.3. Corrective action(s) will be verified by the Monitor.

7.9.4. For resolution of any deviations, issues or queries related to site or investigator procedures, Monitors may not make corrections themselves.

REFERENCES

1. Rogers, H. W., Weinstock, M. A., Feldman, S. R. & Coldiron, B. M. Incidence Estimate of Nonmelanoma Skin Cancer (Keratinocyte Carcinomas) in the US Population, 2012. *JAMA Dermatol* (2015). doi:10.1001/jamadermatol.2015.1187
2. Ravitskiy, L., Brodland, D. G. & Zitelli, J. A. Cost analysis: Mohs micrographic surgery. *Dermatol Surg* **38**, 585–594 (2012).
3. Society, A. C. Cancer Facts & Figures 2015.
4. Firnhaber, J. M. Diagnosis and treatment of Basal cell and squamous cell carcinoma. *Am Fam Physician* **86**, 161–168 (2012).
5. Eide, M. J. *et al.* Relationship of Treatment Delay with Surgical Defect Size from Keratinocyte Carcinoma (Basal Cell Carcinoma and Squamous Cell Carcinoma of the Skin). *J. Invest. Dermatol.* **124**, 308–314 (2005).
6. Russo, T., Piccolo, V., Lallas, A. & Argenziano, G. Recent advances in dermoscopy. *F1000Research* **5**, (2016).
7. Cheng, H. M. & Guitera, P. Systematic review of optical coherence tomography usage in the diagnosis and management of basal cell carcinoma. *Br. J. Dermatol.* **307**, n/a-n/a (2015).
8. Rohrbach, D. J. *et al.* Preoperative Mapping of Nonmelanoma Skin Cancer Using Spatial Frequency Domain and Ultrasound Imaging. *Acad. Radiol.* **21**, 263–270 (2014).
9. Ulrich, M., Astner, S., Stockfleth, E. & Röwert-Huber, J. Noninvasive diagnosis of non-melanoma skin cancer: focus on reflectance confocal microscopy. *Expert Rev. Dermatol.* **3**, 557–567 (2008).
10. Levine, A. & Markowitz, O. In vivo reflectance confocal microscopy. *Cutis* **99**, 399–402 (2017).
11. König, K. Clinical multiphoton tomography. *J. Biophotonics* **1**, 13–23 (2008).
12. Seidenari, S. *et al.* Diagnosis of BCC by multiphoton laser tomography. *Skin Res. Technol.* (2012). doi:10.1111/j.1600-0846.2012.00643.x
13. Batista, A., Breunig, H. G. & König, K. Rapid in vivo vertical tissue sectioning by multiphoton tomography. in *Multiphoton Microscopy in the Biomedical Sciences XVIII* (eds. Periasamy, A., So, P. T., Xie, X. S. & König, K.) **10498**, 39 (SPIE, 2018).
14. Balu, M. *et al.* In Vivo Multiphoton Microscopy of Basal Cell Carcinoma. *JAMA Dermatol* (2015). doi:10.1001/jamadermatol.2015.0453
15. Yew, E., Rowlands, C. & So, P. T. Application of Multiphoton Microscopy in Dermatological Studies: a Mini-Review. *J Innov Opt Heal. Sci* **7**, 1330010 (2014).
16. Zipfel, W. R. *et al.* Live tissue intrinsic emission microscopy using multiphoton-excited native fluorescence and second harmonic generation. *Proc Natl Acad Sci U S A* **100**, 7075–7080 (2003).
17. Gambichler, T., Pljakic, A. & Schmitz, L. Recent advances in clinical application of optical coherence tomography of human skin. *Clin. Cosmet. Investig. Dermatol.* **8**, 345–54 (2015).
18. Sahu, A. *et al.* Evaluation of a Combined Reflectance Confocal Microscopy–Optical Coherence Tomography Device for Detection and Depth Assessment of Basal Cell Carcinoma. *JAMA Dermatology* **154**, 1175 (2018).
19. Hindelang, B. *et al.* Non-invasive imaging in dermatology and the unique potential of raster-scan optoacoustic mesoscopy. *J. Eur. Acad. Dermatology Venereol.* **33**, 1051–1061 (2019).
20. Tang, Z. *et al.* The diagnostic value of in vivo reflectance confocal microscopy in actinic keratosis. *Ski. Res. Technol.* 1–6 (2020). doi:10.1111/srt.12913
21. Esteva, A. *et al.* Dermatologist-level classification of skin cancer with deep neural networks. *Nature* (2017). doi:10.1038/nature21056
22. Reiter, O. *et al.* The diagnostic accuracy of dermoscopy for basal cell carcinoma: A systematic review and meta-analysis. *J. Am. Acad. Dermatol.* **80**, 1380–1388 (2019).
23. Gurcan, M. N. *et al.* Histopathological Image Analysis: A Review. *IEEE Rev. Biomed. Eng.* **2**, 147–171 (2009).
24. König, K. *et al.* Clinical two-photon microendoscopy. *Microsc Res Tech* **70**, 398–402 (2007).
25. Andrew, T. W., Alrawi, M. & Lovat, P. Reduction in Skin Cancer Diagnoses in the UK during

COVID-19 Pandemic. *Clin. Exp. Dermatol.* (2020). doi:10.1111/ced.14411
26. Balu, M., Mikami, H., Hou, J., Potma, E. O. & Tromberg, B. J. Rapid mesoscale multiphoton microscopy of human skin. *Biomed. Opt. Express* (2016). doi:10.1364/BOE.7.004375
27. Giacomelli, M. G. *et al.* Multiscale nonlinear microscopy and widefield white light imaging enables rapid histological imaging of surgical specimen margins. *Biomed. Opt. Express* (2018). doi:10.1364/boe.9.002457
28. Mansouri, B. & Housewright, C. The Treatment of Actinic Keratoses—The Rule Rather Than the Exception. *JAMA Dermatology* **153**, 1200 (2017).
29. Mohan, S. V. & Chang, A. L. S. Advanced Basal Cell Carcinoma: Epidemiology and Therapeutic Innovations. *Curr. Dermatol. Rep.* (2014). doi:10.1007/s13671-014-0069-y
30. Lindelöf, B., Sigurgeirsson, B., Gäbel, H. & Stern, R. S. Incidence of skin cancer in 5356 patients following organ transplantation. *Br. J. Dermatol.* (2000). doi:10.1046/j.1365-2133.2000.03703.x
31. Guy, G. P. *et al.* Prevalence and costs of skin cancer treatment in the U.S., 2002-2006 and 2007-2011. *Am. J. Prev. Med.* **48**, 183–187 (2015).
32. Bichakjian, C. *et al.* Guidelines of care for the management of basal cell carcinoma. *J. Am. Acad. Dermatol.* 1–20 (2018). doi:10.1016/j.jaad.2017.10.006
33. Alam, A. *et al.* Guidelines of care for the management of cutaneous squamous cell carcinoma. *J. Am. Dermatology* **78**, 560–578 (2018).
34. Kauvar, A. N. B., Cronin, T., Roenigk, R., Hruza, G. & Bennett, R. Consensus for Nonmelanoma Skin Cancer Treatment. *Dermatologic Surg.* **41**, 550–571 (2015).
35. Ong, C. S., Keogh, A. M., Kossard, S., Macdonald, P. S. & Spratt, P. M. Skin cancer in Australian heart transplant recipients. *J. Am. Acad. Dermatol.* (1999). doi:10.1016/S0190-9622(99)70525-6
36. Yanik, E. L. *et al.* Spectrum of immune-related conditions associated with risk of keratinocyte cancers among elderly adults in the United States. *Cancer Epidemiol. Biomarkers Prev.* (2017). doi:10.1158/1055-9965.EPI-17-0003
37. Codazzi, D. *et al.* Positive compared with negative margins in a single-centre retrospective study on 3957 consecutive excisions of basal cell carcinomas. Associated risk factors and preferred surgical management. *J. Plast. Surg. Hand Surg.* (2014). doi:10.3109/2000656X.2013.800526
38. Campanella, G. *et al.* Clinical-grade computational pathology using weakly supervised deep learning on whole slide images. *Nat. Med.* (2019). doi:10.1038/s41591-019-0508-1
39. Sanchez, G. N. *et al.* In Vivo Imaging of Human Sarcomere Twitch Dynamics in Individual Motor Units. *Neuron* **88**, 1109–20 (2015).
40. Lichtwark, G. A., Farris, D. J., Chen, X., Hodges, P. W. & Delp, S. L. Microendoscopy reveals positive correlation in multiscale length changes and variable sarcomere lengths across different regions of human muscle. *J. Appl. Physiol.* japplphysiol.00480.2018 (2018). doi:10.1152/japplphysiol.00480.2018
41. Chen, X. & Delp, S. L. Human soleus sarcomere lengths measured using in vivo microendoscopy at two ankle flexion angles. *J. Biomech.* (2015). doi:10.1016/j.jbiomech.2016.11.010
42. Chen, X., Sanchez, G. N., Schnitzer, M. J. & Delp, S. L. Changes in sarcomere lengths of the human vastus lateralis muscle with knee flexion measured using in vivo microendoscopy. *J. Biomech.* (2016). doi:10.1016/j.jbiomech.2016.07.013
43. Rogers, H. W. *et al.* Incidence estimate of nonmelanoma skin cancer in the United States, 2006. *Arch Dermatol* **146**, 283–287 (2010).
44. Hansen, C., Wilkinson, D., Hansen, M. & Argenziano, G. How good are skin cancer clinics at melanoma detection? Number needed to treat variability across a national clinic group in Australia. *J. Am. Acad. Dermatol.* **61**, 599–604 (2009).
45. Wilson, L. S. *et al.* Fee comparisons of treatments for nonmelanoma skin cancer in a private practice academic setting. *Dermatologic Surg.* **38**, 570–584 (2012).
46. González, S. & Gilaberte-Calzada, Y. In vivo reflectance-mode confocal microscopy in clinical

dermatology and cosmetology. *Int. J. Cosmet. Sci.* **30**, 1–17 (2008).
47. Levine, A. & Markowitz, O. Introduction to reflectance confocal microscopy and its use in clinical practice. *JAAD Case Reports* (2018). doi:10.1016/j.jdcr.2018.09.019