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.