Executive Summary
A fully scalable optical tissue spectroscopy solution has been developed for a wide range of applications to improve healthcare diagnostics and outcomes. As a result, health practitioners can now implement quantitative tissue spectroscopy can help them improve health outcomes in their field of work.

The Zenascope™ Quantitative Optical Spectrometer uses standard spectroscopic measurement hardware, proprietary software, and patented algorithms to achieve rapid, quantitative, and non-destructive analysis of biological tissue characteristics that reflect the underlying function and composition of the tissue.

A number of pre-clinical and clinical studies carried out at the Duke University Medical Center have already demonstrated the efficacy of this quantitative diagnostic technique. Application areas include: accelerating feedback in drug discovery; tumor margin assessment; assessment of response to cancer therapy; as well as screening and diagnostic applications in breast, cervical and head and neck cancer.

Figure 1. The Zenascope system, software output and an example measurement in which extracted hemoglobin saturation (the percentage of oxygenated hemoglobin to total hemoglobin content) is shown relative to expected values.
Spectroscopy in Biological Tissue: How it Works

The Zenascope™ (Figure 2) is a uv-NIR (ultraviolet-near-IR) spectrometer that uniquely achieves quantitative optical spectroscopy in turbid media. The system is a specialized, real-time, measurement device that shines white light on opaque target media and then measures and analyzes the reflected signal. Proprietary algorithms\(^1\) and standardized measurement hardware achieve rapid, quantitative analysis of optical properties related to targeted endpoints. This novel approach enables a host of new applications for visible spectroscopy in non-ideal, scattering conditions.

In a first target application, the Zenascope is being used as a biological tissue spectrometer to reliably, quickly and non-destructively measure important biological tissue characteristics that reflect underlying function and composition (Figure 3). Using these quantitative endpoints, the device can detect and monitor the presence of cancer as well as other disease states. The depth of penetration of the optical signal is approximately 2-3 mm, depending on tissue type.

The Science Behind Optical Tissue Spectroscopy

In diffuse optical spectroscopy, wavelengths of interest span the UV-NIR spectral range – from the ultraviolet (UV) at ~300 nanometers through to the beginning of near-infrared (NIR) at ~650 nanometers – a region which is sensitive to the optical absorption and scattering of soft tissues. The shape and magnitude of the absorption depends on the concentration of the dominant tissue absorbers as well as

their extinction coefficient (an inherent measure of a constituent’s ability to absorb light energy). In biological tissue, absorbers of interest include oxygenated hemoglobin (HbO₂) and deoxygenated hemoglobin (dHb), beta-carotene, melanin, and proteins in the UV-NIR spectrum. Since diffuse reflectance spectroscopy can measure both HbO₂ and dHb, one can estimate both the total blood concentration (THb = HbO₂ + dHb) and the percent oxygenation saturation (SO₂ = 100xHbO₂/THb).

Furthermore, the optical scattering coefficient is known to be sensitive to the spatial architecture and organization of the tissue and therefore can be used as a means to track changes in cellular morphology and density, in particular proliferation or necrosis.

Once measured, the diffuse reflectance must be processed through rigorous computational models to obtain quantitative information about the absorption and scattering properties of the tissue. The Zenalux algorithm uses a fast, Monte Carlo approach that has been developed to extract quantitative absolute optical properties from diffuse reflectance spectra by employing scaling and similarity relationships that accelerate the modeling. In short, the Zenalux algorithm quickly compares the measured reflectance spectra to spectra generated using the Monte Carlo model; when the modeled and experimental reflectance spectra match, the underlying optical properties of the medium are determined. Once absorption (μₐ) is determined, concentration of the absorber can also be determined through the Beer-Lambert law. This forms the very basis of quantitative optical tissue analysis using the Zenalux Zenascope.

An extensive series of studies have been conducted to test the validity of our model in quantitatively extracting hemoglobin oxygen saturations, total hemoglobin concentrations, as well as the optical scattering coefficients. In these studies, diffuse reflectance spectra were obtained using well-controlled, tissue-simulating phantoms. The performance of the algorithm was tested by measuring the diffuse reflectance data from liquid tissue phantoms that were prepared by diluting stock solutions of hemoglobin (as the chromophore absorber) and polystyrene microspheres (to simulate mismatches in refractive index that cause scattering in tissue) to a final fixed volume. Knowledge of the stock concentrations of hemoglobin and scatterer solutions allowed calculation of the expected optical properties of absorption and scattering in the final phantom. Hemoglobin desaturation was achieved by adding yeast (which consume oxygen) and measuring the partial pressure of dissolved oxygen using an oxygen electrode. The measured diffuse reflectance was analyzed using our inverse Monte Carlo model in order to extract optical absorption and scattering from the phantoms (Figure 4).
Figure 4: Expected and extracted results achieved with the Zenalux Algorithm in turbid suspensions containing varying concentrations of hemoglobin and polystyrene microspheres. (A) Zenascope measured hemoglobin saturation (%HbO2) is shown relative to expected values. (B) Zenascope measured scattering (µs') is shown relative to expected scattering.

Ease of Use: The Key to Implementation
The implementation of UV-NIR spectroscopy in healthcare has been hampered by the fact that healthcare practitioners are typically not spectroscopists and specialists in spectroscopy are not generally practicing physicians. The Zenascope is changing that by offering a simple-to-use, inexpensive, quantitative optical spectroscopy platform that requires no prior knowledge of spectroscopy techniques for successful implementation.

The base-line Zenascope assesses the following endpoints:
- Average scattering (µs'), which is related to cellular density, necrosis and fibrous content;
- Total hemoglobin (THb) content, relating to vascularity; and
- Hemoglobin saturation (HbO2), relating to vascular oxygenation and/or hypoxia.

An example of the Zenascope output is shown in Figure 5.
Custom Implementation
A very important aspect of tissue spectroscopy is that different tissue environments have different dominant absorbers, in addition to THb and HBO₂, and these need to be accounted for in the spectral analysis. For example: in head and neck cancer studies, skin and melanin have to be accounted for. In breast cancer studies, beta-carotene (found in fat) has to be accounted for. In some cases, absorbers are added as part of therapy, and these also have to be accounted for.

Figure 6 shows results for in vitro validation of a target drug using known stock solutions and polystyrene microspheres to provide scattering medium. In this case, the diffuse reflectance was measured and spectra were analyzed using the Zenalux algorithm with drug absorption coefficients and concentrations extracted in real-time. The result was a measured absorption error <10% with a linear absorption range up to at least 6 cm⁻¹. It should be noted that this represents a greater linear absorption dynamic range than most research spectrophotometers using non-turbid (i.e. clear) samples.

Such validation measurements often take less than a day to implement, and with these one-time measurements it is possible to configure the Zenascope to measure the target drug for future use, in addition to total hemoglobin, hemoglobin saturation and scattering.

Accounting for all major absorbers is critical for accurate, quantitative results. Figure 7 shows the spectral fit for Zenascope tumor measurements before and after adjusting the Zenalux algorithm for a dominant absorber/scatterer in the skin of the subject mice.
Figure 7: (A) Accounting for only THb, Hb saturation and scattering in this mouse model results in error. (B) The Zenalux algorithm can easily account for absorption and scattering in the hair follicles of the mouse. This is a critical step in achieving quantitative results.

Technology Impact

Various completed and ongoing preclinical and clinical studies at Duke University Medical Center are consistently demonstrating the promise held by this novel, non-invasive and quantitative technology to measure physiological changes across a variety of organ sites. Experiments have demonstrated a clear concordance between the optical measurements of oxygen saturation and physiological endpoints of tumor pO$_2$ [1] and immunohistochemical markers of hypoxia [2]. In separate studies the same technology was used to measure cancer vascularity as ascertained by immunohistochemical methods [3].

Even further studies have shown how these techniques may provide insight into changes in tumor physiology as the animals were exposed to radiation and chemotherapeutic treatments [4, 5]. In a study published by Vishwanath et al [4] oxygen saturation measured from a murine model of head and neck cancer was able to predict partial response vs. local control in animals treated with a single dose of radiation as early as 7 days after treatment initiation (see Figure 8) [2]. In another study published by Palmer et al [5] the baseline oxygen saturation was inversely related to tumor growth rate after treatment with low temperature liposomal Doxorubicin. In these studies it was found that the optical endpoint of vascular oxygen saturation predicted treatment outcomes many weeks before volumetric measurements indicated these outcomes. These preliminary findings are particularly interesting as they indicate the potential of obtaining longitudinal measurements from the individual subjects, thereby tracking how the underlying tumor physiology changes during treatment, which in turn could provide insights to identify individual response.
Figure 8. Zenascope probe measurements of pre-clinical head and neck tumors treated with radiation and matched controls. (A) Tumor volume shows similar reduction in volumes in tumors that achieve local control (CR) and those that recur at a later time point (PR). (B) However, tumor oxygen saturation shows a statistically significant increase in the CR group which is not observed in the PR group leading to a statistical difference in the oxygen saturation between complete and partial responders that is strongly associated with outcome[4].

In addition, published studies have also examined tumor oxygen saturation within the tumor microenvironment in vivo in the breast (see Brown et al.[6]), where changes in the tumor oxygenation patterns were observed between different molecular subtypes of breast cancer (see Figure 9). Overall, malignant breast tissues were found to have lower oxygen saturation relative to normal breast tissue, but there was a clear variation in the oxygenation pattern of the tumors dependent on the molecular sub-type of the tissue itself.

Figure 9. A) Zenascope probe adapted to fit a breast biopsy needle. B) Ultrasound image of probe in breast. C) Box plots of vascular hemoglobin saturation measured in the breast in vivo, stratified by pathologic diagnosis of the corresponding tissue biopsy.

The Zenalux technology has been employed in the cervix to elucidate the underlying sources of absorption and scattering contrast in 39 patients[7]. The results of this study showed a significant increase in absorption (Figure 10) and total hemoglobin content in CIN 2+ (high grade lesions) compared to normal and CIN 1 (low grade dysplasia/benign tissue). This has also been validated independently with immunohistochemical staining of endothelial cells, which demonstrated that microvessel density
(representative of neovascularization) was statistically higher in CIN 2+ tissues compared to CIN 1 and normal tissues[3].

**Figure 10.** Immunohistochemical staining of endothelial cells highlighting micro vessels in brown and corresponding absorption spectra of CIN 2+, CIN 1 and normal tissues[3].

In the breast, the Zenalux technology has been proven to be sensitive to sources of intrinsic optical contrast, in particular, hemoglobin, beta-carotene and scattering which effectively discriminate diseased from healthy tissues for intra-operative margin assessment (see Zhu et al.[8-10], Palmer et al.,[11-14] Wilke et al.,[15] Brown et al.[16] and Figure 11).

**Figure 11.** 49-channel probe and excised breast specimen in specimen holder (green dots indicating margin) and resulting parameter maps of beta carotene to scattering for a positive and negative margin[15].

A variety of implementation modes have been demonstrated: from *ex vivo* analysis of margin specimens after removal from the breast (Figure 11), to *in vivo* tissue analysis conducted through the lumens of biopsy needles and cannulas (Figure 9). Furthermore, the Zenalux technology has been demonstrated as a useful tool for measuring physiological endpoints pertinent to tumor response to therapy.
Zenascope Summary of Benefits

- **Speed of use**
  - Measurement takes less than 1 second; results are immediate
- **Non-destructive measurement**
  - Incident light (white light) is non-harmful
  - Tissue does not need to be removed for analysis, enabling harmless monitoring over time in the same animal
- **Quantitative analysis**
  - Algorithm quantifies biomarker concentrations
- **Flexibility**
  - Additional absorbers that could interfere with analysis are easily accounted for in the algorithm
- **Cost effectiveness**
  - Real-time, nondestructive monitoring significantly reduces cost of analysis
- **Ease of use**
  - Set-up and implementation take less than five minutes; no special training is required

**Future Work**
The goal at Zenalux is to develop the Zenascope as a fully scalable optical tissue spectroscopy solution for a wide range of applications to improve healthcare diagnostics and outcomes. Our mission is to work closely with any health practitioner who feels that quantitative tissue spectroscopy can help them improve health outcomes in their field of work.

For more information, visit [www.zenalux.com](http://www.zenalux.com).
References


