Adding an Additional Absorber to the Zenascope™
Zenalux Biomedical, Inc.

A customer approached Zenalux with the need to measure a drug concentration in tissue, along with hemoglobin concentration, hemoglobin saturation and scattering. Requirements included high-speed, in vivo analysis in order to monitor drug delivery in real time.

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. In its standard configuration, the Zenascope is set up to measure hemoglobin, blood saturation and scattering in real time. More importantly, however, the Zenascope can easily be custom configured to measure other biomarkers that absorb light in the visible (white light) spectrum. In this case, the target drug had an excellent absorption cross section and the goal was to demonstrate the ability to measure the drug at very low (i.e., clinically relevant) concentrations.

Figure 1: The Zenascope system, software output and an example measurement demonstrating the capability of the Zenascope. In the demonstration, hemoglobin saturation (the percentage of oxygenated hemoglobin relative to total hemoglobin content) measured by Zenascope was compared to oxygen pressure (pO₂) measured by an invasive technique while oxygen was controllably depleted.
Experiment
Zenalux first validated hemoglobin, hemoglobin saturation and scattering measurements using the Zenascope™.

In vitro validation was completed using known stock solutions of hemoglobin, oxygenated hemoglobin and polystyrene microspheres. The oxygenated hemoglobin solution was de-saturated using yeast and pO₂ was measured using an oxygen microelectrode. Using the Zenascope system and Zenalux algorithm, diffuse reflectance was then measured and spectra were analyzed.

When measured results were compared to expected results, measured absorption error was <3% and measured scattering error was <5%. Importantly, the system demonstrated the ability to track changes in hemoglobin saturation (as validated against the pO₂ electrode), which indicates the utility for monitoring vascular oxygenation in tissue non-invasively.

In addition to these available measurements, the customer also needed to measure a target drug concentration in tissue in vivo. Consequently, an additional absorber validation was carried out. In vitro validation was completed using known stock solutions of the target drug, along with polystyrene microspheres and hemoglobin to provide a tissue-like scattering medium. Diffuse reflectance was measured using the Zenascope and spectra were analyzed using the Zenalux algorithm. Drug absorber coefficients and concentration were extracted in real-time.
Figure 4: Peak drug absorbance (at 658 nm) measured using the Zenascope, compared to known values. In addition to drug concentration, scattering coefficients and hemoglobin concentration were extracted from the Zenascope measurement as well.

When measured results were compared to expected results, measured absorption error was <10% and linear absorption range was up to at least 6cm⁻¹. Remarkably, the Zenascope™ provided a greater linear absorption dynamic range than most benchtop research spectrophotometers operating in non-turbid (i.e. clear) samples are capable of. Importantly, these results demonstrate how the customer can monitor the peak absorbance (and thus concentrations) of their target drug in tissue non-invasively and in real time over a large range of concentrations, even in the presence of other biological absorbers (i.e. hemoglobin).

**Zenascope Spectroscopy in Biological Tissue: How it Works**

The Zenascope is a VIS-NIR (visible-near-infrared) 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 algorithms¹ 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 diffuse optical spectroscopy, wavelengths of interest span the VIS-NIR spectral range – from the visible (VIS) at ~455 nanometers through to the near-infrared (NIR) at ~800 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 VIS-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 ($\mu_a$) 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™.

**Zenascope™ Configurations**

The Zenascope system is offered in both standard and custom configurations. The standard Zenascope system configuration offers quantitative measurement of hemoglobin concentration, hemoglobin saturation and scattering. Two software configurations are offered: Zenaware™ Discovery—a spectral analysis and real-time data recording in an electronic record management system; and Zenaware Monitor—a spectral analysis and real-time monitoring on a simple, high visibility screen.

**Figures 5 and 6:** Screen captures of Zenaware Discovery and Zenaware Monitor software output, respectively.

For the custom Zenascope configuration, the Zenaware Monitor interface was modified to include the new absorber. As a result, the Zenascope now provides quantitative measurement of hemoglobin concentration, hemoglobin saturation, scattering and drug concentration.
Summary
As evidenced in the data from this study, Zenascope™ can easily be configured to include custom absorbers and biomarkers of choice. A number of benefits are realized using the Zenascope system, including:

1. Speed – Measurement takes less than one second and results are immediate;
2. Non-destructive – Incident light (white light) is non-harmful, and the system does not require removal of tissue and destructive assays to measure drug concentration (for example, as in HPLC analysis);
3. Quantitative – Zenalux’s patented algorithm quantifies biomarker concentrations;
4. Flexible – Additional absorbers that could interfere with analysis are easily accounted for in the algorithm;
5. Cost effective – Real-time, non-destructive monitoring significantly reduces cost of analysis;
6. Ease-of-use – Set-up and implementation takes less than five minutes, and no special training is required.

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. Zenascope is currently designated for investigational purposes only.

For more information on the Zenascope, visit www.zenalux.com or call +1- 919-794-5757.