Monitoring Tumor Therapy

Zenalux Biomedical, Inc.

The Zenascope™ was used to monitor the physiological response to a vascular disrupting agent in mouse tumors. Changes in hemoglobin concentration, hemoglobin saturation and scattering (a measure of cell density) were monitored non-destructively during a 24 period after the drug was administered.

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.

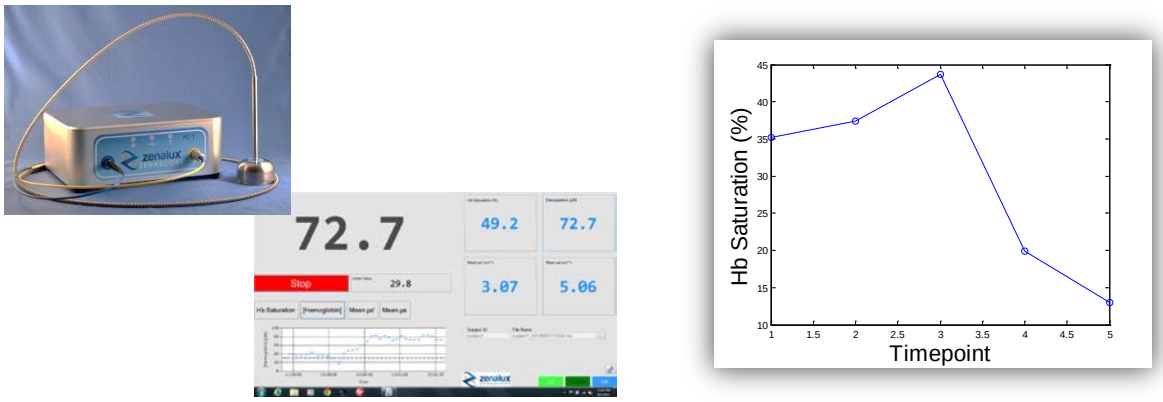


Figure 1: The Zenascope system, software output and an example measurement in which extracted hemoglobin saturation (the percentage of oxygenated hemoglobin to total hemoglobin content) was monitored in a subject at various time points during a 24 hour period.

Experiment

Zenalux conducted a study that was designed to use the Zenascope (Figure 2) to assess the impact on tumor pathophysiology of blood vessel-directed anti-cancer therapies.

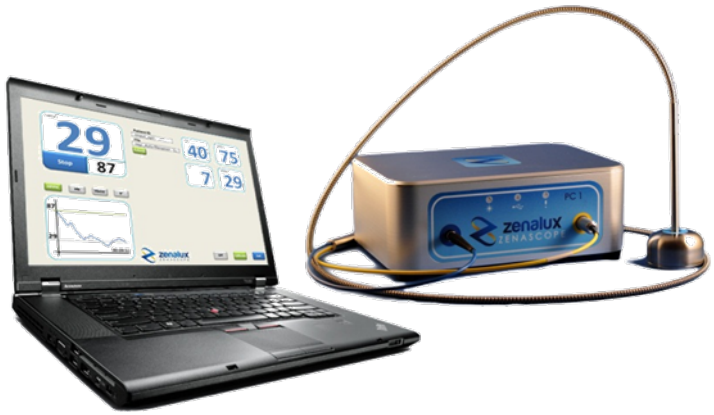


Figure 2: The Zenascope™

Expected Response

In previous experiments[1] (Figure 3), animals were sacrificed and patent blood vessels were counted on histological sections of removed tumors. Patent blood vessels were found to be sharply decreased as a result of treatment at the four-hour time point.

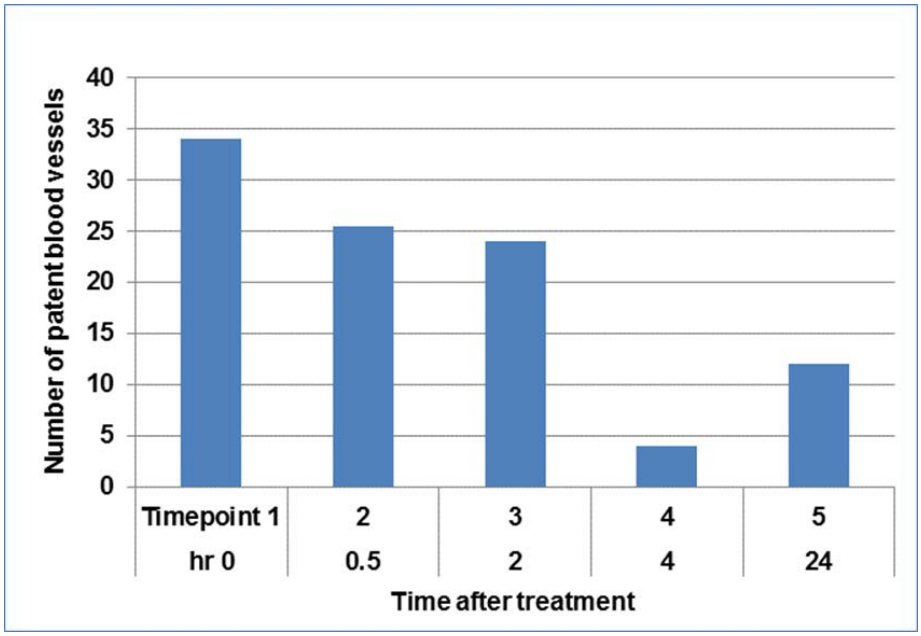


Figure 3: Expected response based on actual patent blood vessel count from previous, destructive analysis

[1] Howard W. Salmon and Dietmar W. Siemann, Clin Cancer Res 2006;12(13) July1, 2006

Using the Zenascope

Monitoring of the cancer therapy was repeated using the Zenascope. A vascular disrupting agent was administered to 17 mice (Table 1). Changes in hemoglobin concentration, hemoglobin saturation and scattering (a measure of tissue morphology) were monitored using the Zenascope.

Table 1: Zenascope experimental procedure for monitoring tumor therapy

Time Point #	Time (hr)	Action	Zenascope Measurement (Hb, HbO_2, $\mu_{s'}$)
1	-0.5		Sample all sites all animals
-	0	Administer drug	
2	0.5		Sample all sites all animals
3	2		Sample all sites all animals
4	4		Sample all sites all animals
5	24		Sample all sites all animals

A schematic of the Zenascope experiment is shown in Figure 4. The depth of penetration of the optical signal was 2-3mm. The probe was placed at multiple sites on the tumor and measurements were collected in less than one second. For each measurement, the following biomarkers were quantified, displayed and stored: hemoglobin content; hemoglobin oxygenation; and scattering levels.

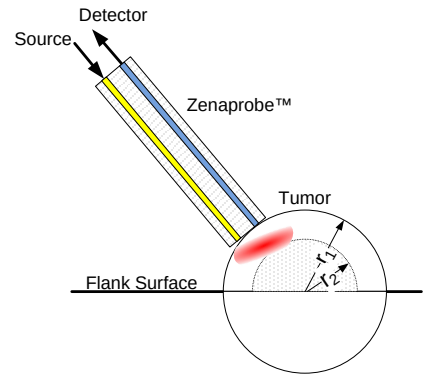


Figure 4: Diagram of non-invasive, real-time diagnostic technique

Zenascope 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[2] 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 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 (HbO_2) and deoxygenated hemoglobin (dHb), beta-carotene, melanin, and proteins in the UV-NIR spectrum. Since diffuse reflectance spectroscopy can measure both HbO_2 and dHb, one can estimate both the total blood concentration (THb = HbO_2 + dHb) and the percent oxygenation saturation (SO_2 = 100xHbO_2/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 (μ_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.

Results

Figure 5 depicts representative spectroscopic data captured by the Zenascope during the experiment. The Zenascope algorithm successfully tracked the change in physiology in the subject throughout the experiment resulting in model results that were completely conistent with the measured data.

Based on the model results, quantative measurements of scattering, hemoglobin concentration, and hemoglobin oxygen saturation were derived by the Zenascope (Figure 6). Scattering intially increased several-fold post-treatment and then returned to baseline at timepoint four (four hours after treatment). Hemoglobin showed dramatic increase starting at timepoint four (four-hours after treatment) while hemoglobin oxygenation showed a modest increase up to hour three, then dramatically decreased afterward, tracking increase in Hb concentration.

[2] US Patent #7,570,988, Method for Extraction of Optical Properties from Diffuse Reflectance Spectra, N. Ramanujam, Greg Palmer.

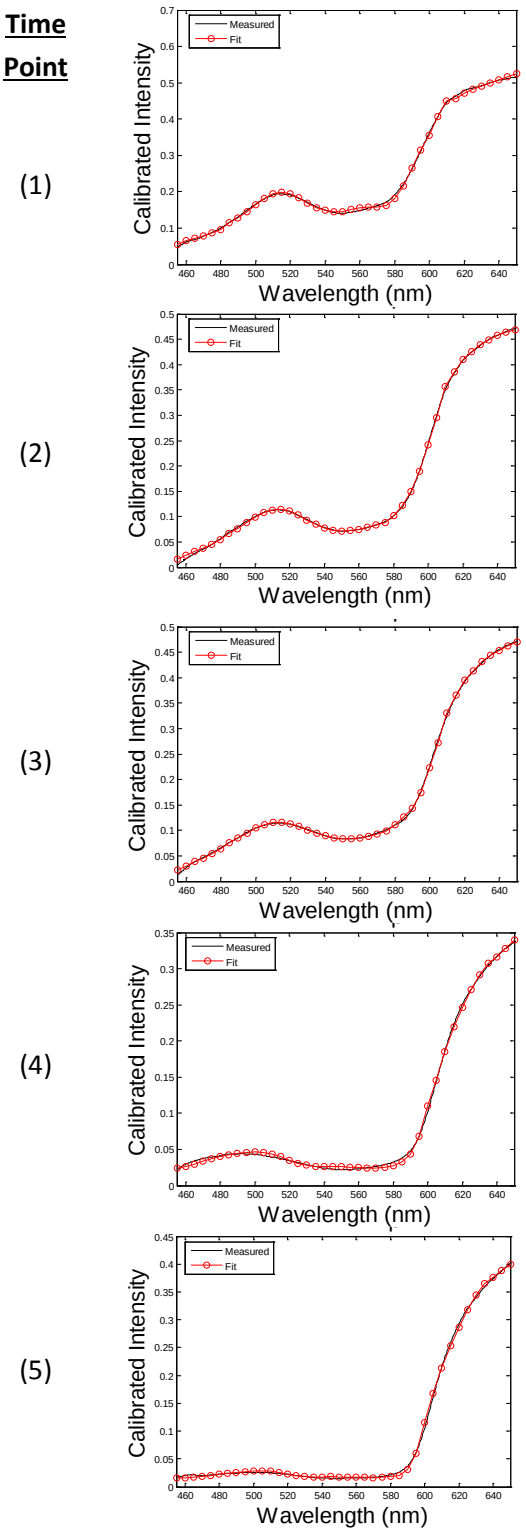


Figure 5: Spectra and Zenascope data fit for one of the subjects for time points 1-5 listed in Table 1. Data fits are derived using Zenalux's algorithm.

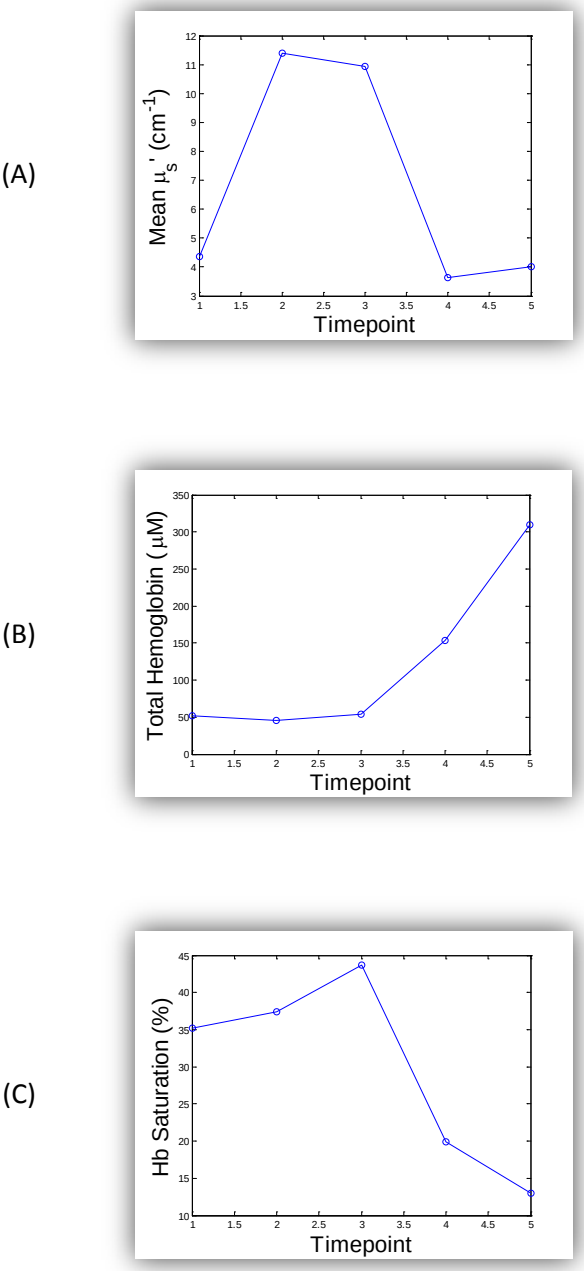
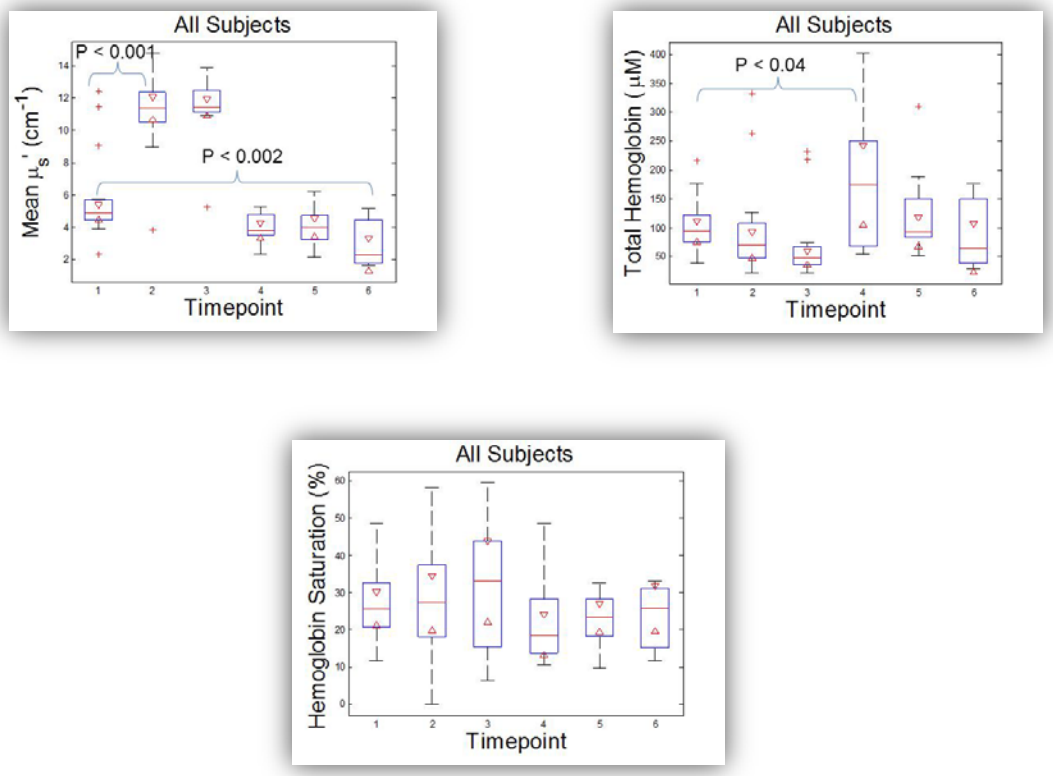


Figure 6: Quantitative results output by the Zenascope based on the spectra and data fits in Figure 6. (A) scattering, (B) hemoglobin, and (C) hemoglobin oxygenation at time points outlined in Table 1.

Results were recorded for all 17 animals for statistical analysis (Figure 7). Scattering effects could be linked to apoptosis, necrosis and cell invasion. Total hemoglobin content likely increased due to hemorrhage after vascular disruption at the four-hour time point. These results are consistent with the disruption of patent blood vessels observed at the same time point in the destructive, histologic assay.



(A) (B)

(C)

Figure 5: Zenascope quantitative results (A) scattering, (B) total hemoglobin concentration and, (C) hemoglobin saturation for all animals. Note P-values given are Wilcoxon rank sums

Summary

As evidenced in the data from this study, Zenascope is an effective tool for real-time monitoring during tumor therapy. The device effectively and non-destructively monitors key biomarkers in real time. 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 tissue does not need to be removed for analysis, enabling harmless longitudinal monitoring over time in the same animal;
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 health practitioners and basic researchers who feel 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.