# Monitoring of Head and Neck Tumor Physiology in Response to Hyperoxia and Hypoxia using a Side-firing Fiber Optic Probe

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## Abstract

A multi-wavelength frequency-domain photon migration (FDPM) instrument with a side-firing fiber optic probe was used to quantify tumor oxygenation and hemoglobin concentrations in nude rats bearing human head & neck (H&N) (FaDu) tumors during normoxia, hyperoxia and cyclic hypoxia. Significant increase (with carbogen gas breathing) or decrease (with reduced $O_2$ supply) in tumor oxygenation was observed. The studies demonstrated the feasibility of the technology for longitudinal monitoring of H&N tumor's response to therapy.

## Motivation

- Routine practice for H&N tumor includes diagnosis with one or a combination of CT, PET, MRI and panendoscopy, followed by surgical biopsies and treatment by radiotherapy, chemotherapy, and/or surgery[1].
- Obtaining a biopsy during a clinical exam can be technically challenging, uncomfortable for the patient, costly due to the need of pathology, and can cause complications, such as infection and damage to the organ.
- Current method for evaluating the outcome of the cancer therapy is based on measuring the tumor size for several weeks to months[2,3]. Significant delay in switching to an alternative treatment could happen to the non-responders.
- Recent studies, including those by our group[3], have demonstrated the potential of UV-VIS optical spectroscopy in helping diagnosis and treatment monitoring of H&N tumors.
- H&N cancers often spread to the lymph nodes in the neck and a swelling neck node a few millimeters to centimeters under the skin is common. Neck nodes have been studied for H&N tumor detection and staging[4], and more recently for evaluating treatment outcomes[5,6].
- The advantage of using neck nodes for both diagnosis and therapeutic monitoring is that a large neck node can occur earlier than a primary lesion can be identified and it exists to the end of all treatments, making it an ideal site for longitudinal assessment using optical spectroscopy.
- Major challenges for using UV-VIS optical spectroscopy for neck nodes are: small penetration depth and difficulty in reliably placing a probe for a long period, thus introducing large random errors due to operator bias.
- We report the use of FDPM and a flat side-firing probe[7] for quantifying tumor physiology in response to hyperoxic and cycling hypoxic gas breathing in a preclinical model.

## Instrument

An FDPM instrument with 6 lasers (654, 683, 779, 805, 847, and 905 nm) and a side-firing probe has been developed for the studies[7]. The flat probe design makes it easily attached on a flat surface. The probe has two source detector separations (SDS=5 and 10 mm). The instrument launches intensity-modulated lasers into the tissue and collects the amplitude-attenuated and phase-shifted diffuse reflectance from the tissue at the same frequency. The modulation frequency was scanned from 50-250 MHz at a 1 MHz interval at each wavelength and SDS combination.

The relative amplitude attenuation ($Att_{ac}$) was obtained by dividing the AC amplitude of the long SDS ($r_2$) by that of the short SDS ($r_1$) and the relative phase-shift ($\Delta\phi_{ac}$) was calculated as the difference between the phases of the two SDSs. The absorption ($\mu_a$) and reduced scattering coefficients ($\mu_s$') at each wavelength were extracted from $Att_{ac}$ and $\Delta\phi_{ac}$ using a diffusion approximation model in the frequency domain for semi-infinite medium[8] and nonlinear least square fitting.

$$Att_{ac}(\lambda_i,\omega) = U_{ac}(\lambda_i,\omega,r_2)/U_{ac}(\lambda_i,\omega,r_1)$$
$$\Delta\phi_{ac}(\lambda_i,\omega) = \phi(\lambda_i,\omega,r) - \phi(\lambda_i,\omega,r)$$

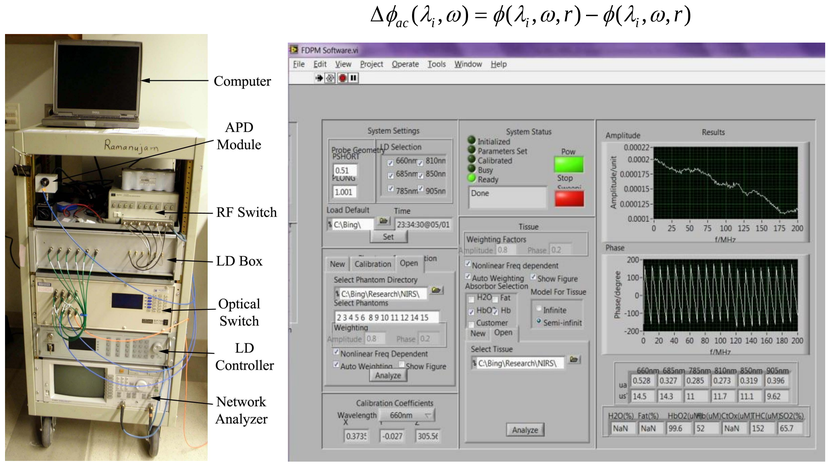



Fig.1. FDPM instrument and LabVIEW user interface.

## Phantom Validation

15 tissue-simulating liquid phantoms with $\mu_a$ and $\mu_s$' representative of rat tumors were used to evaluate the performance of the system and for instrument calibration.

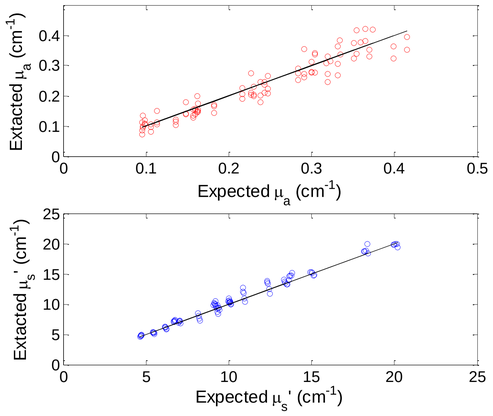


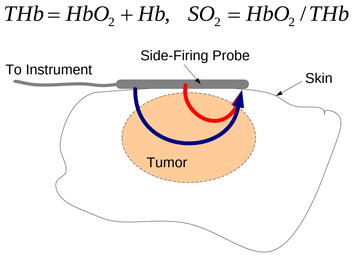

Fig.3. Extracted v.s. expected $\mu_a$ and $\mu_s$' at all wavelengths for all phantoms. Phantoms were obtained with fixed number of scatterers (20% Intralipid) at 3 levels and titration of India ink (as absorbers) to 5 levels. $\mu_a$ and $\mu_s$' were independently characterized using a diffuse reflectance setup and a spectrophotometer, respectively.

## Animal Studies

A human H&N tumor model (FaDu) in athymic nude rats (Charles River) was used. The study was approved by the Duke IACUC.

- A total of 22 rats: 8 with tumor for hyperoxia study, 10 with tumor and 4 normal controls for cycling hypoxia study.
- 5M FaDu cells were injected into the left flank of each rat (200-240g), and the tumors reached ~1.5-2.0 cm in diameter in 2-3 weeks.
- Rats were anesthetized with 50 mg/kg Pentobarbital.
- A Starr Mouse-Ox pulse oximeter was attached to the left foot as a reference in the cycling hypoxia study.
- Tissue hemoglobin concentrations and oxygenation were calculated by:

$$\mu_a(\lambda_i) = 2.303\cdot(\varepsilon_{(HbO_2,\lambda_i)}\cdot HbO_2 + \varepsilon_{(Hb,\lambda_i)}\cdot Hb)$$
$$THb = HbO_2 + Hb,\quad SO_2 = HbO_2/THb$$



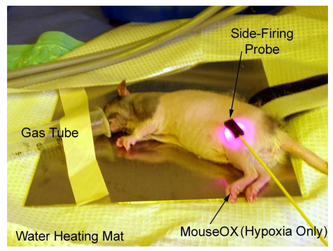




Fig.2. Experimental setup for carbogen and hypoxia gas breathing.

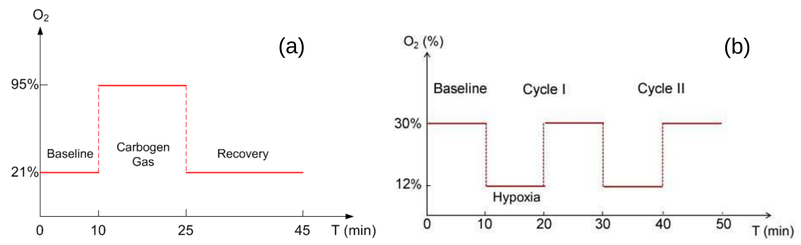



Fig.4. Experimental procedures for (a) carbogen and (b) cycling hypoxia gas breathing. 30% $O_2$ was used for normoxia in the hypoxia study.

## Animal Experiment Results

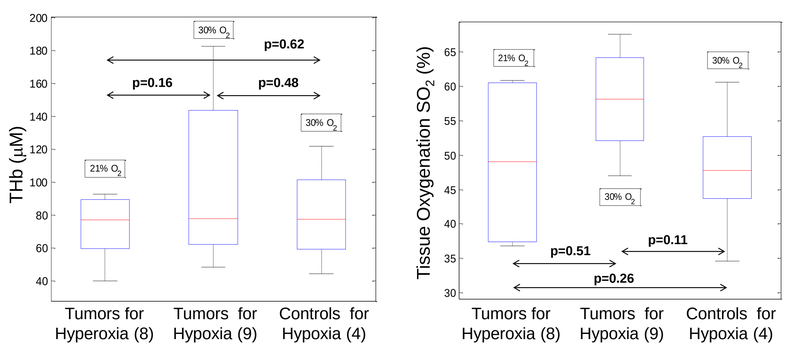



Fig.5. Boxplots of baseline THb and $SO_2$ for tumors for hyperoxia and hypoxia studies and normal controls for the hypoxia study.

***Acknowledgement:*** *The authors would like to thank Drs. Narasimhan Rajaram and Yulin Zhao for providing the cell lines and Drs. Mark Dewhirst and Thies Schroeder for providing the animal experiment setups. This work was supported by the DOD BCRP Era of Hope Scholar award to Dr. Nirmala Ramanujam.*

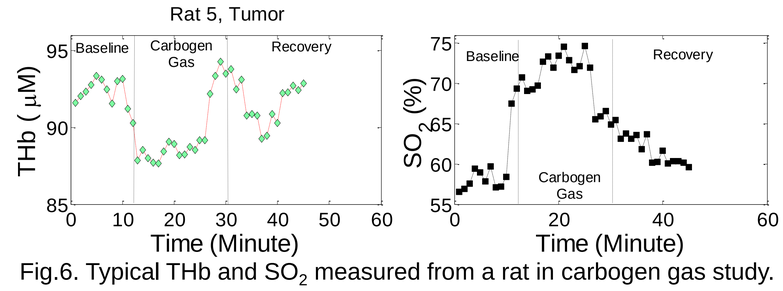



Fig.6. Typical THb and $SO_2$ measured from a rat in carbogen gas study.

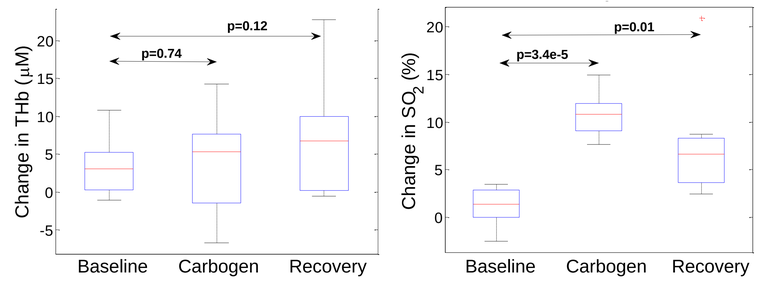



Fig.7. Boxplots of change in THb and $SO_2$. Significant increase in $SO_2$ from baseline was observed during Carbogen breathing. The $SO_2$ did not fully return to baseline in 20 minutes. Changes in THb were not significant.

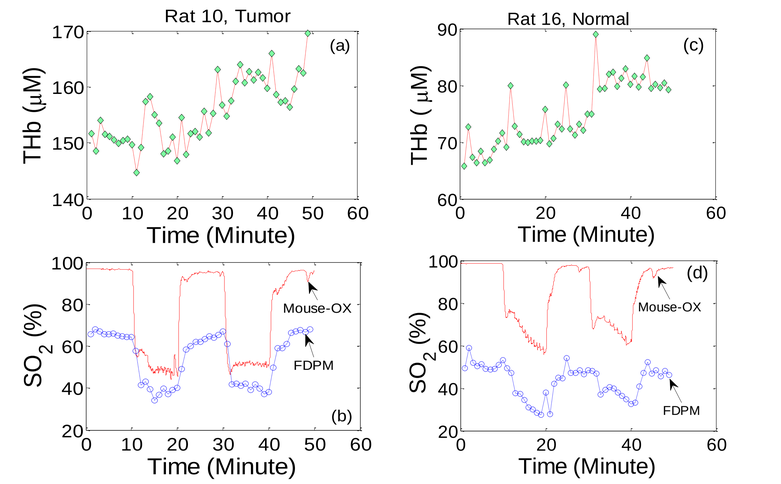



Fig.8. Typical THb and $SO_2$ measured in the cycling hypoxia study from a rat tumor ((a) and (b)) and a normal control ((c) and (d)).

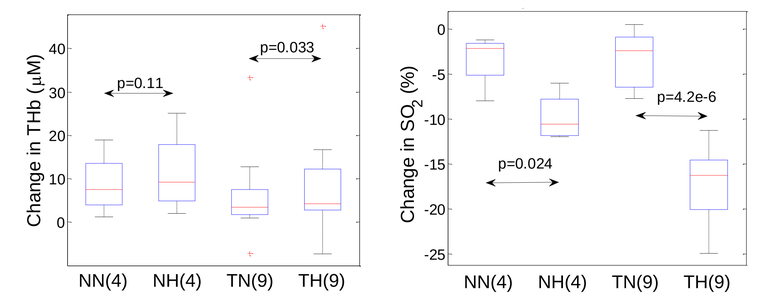



Fig.9. Boxplots of changes in THb and $SO_2$ for normal normoxia (NN), normal hypoxia (NH), tumor normoxia (TN) and tumor hypoxia (TH). Significant differences in $\Delta SO_2$ were found between normoxia and hypoxia for both normal and tumorous rats. $\Delta$THb was only significant for tumors.

## Summary

We have developed a frequency-domain photon migration technology and a side-firing fiber optic probe that can be used to quantify tissue optical and physiological parameters. The flat design of the side-firing probe makes it easily attachable to any flat surface. The liquid phantom experiment indicated that the technology can quantify tissue absorption and scattering coefficients with good accuracy. The *in vivo* rat study with hyperoxic and hypoxic gas breathing demonstrated the potential of the technology for longitudinal monitoring of H&N tumor physiological changes in response to treatment.

## References

1) NCI factsheet: http://www.cancer.gov/cancertopics/factsheet/Sites-Types/head-and-neck.
2) Doweck I, et al, Laryngoscope. 2002 Oct;112(10):1742-9
3) Vishwanath K, et al., J Biomed Opt. 2009;14(5):054051. PMCID: 2776819.
4) Lacau St Guily JL. Rev Prat. 2006 Oct 15;56(15):1652-7.
5) Sunar U, et al., J Biomed Opt. 2006;11(6):064021
6) Furukawa MK and Furukawa M. Int J Clin Oncol. 2010;15(1):23-32
7) Yu B, et al., ,Optics Express, vol. 15, no. 12, 2007, pp. 7335-50.
8) S. Fantini S, et al., J Opt Soc Am B. 1994;11:2128-38.
9) R.C. Haskell et al., J Opt Soc Am A. 1994;11:2727-41.