Monitoring photochemistry in tissue using frequency domain photon migration

Tsong-Tseh Tsay[1], Bruce J. Tromberg[1], Eric H. Cho[1], Khai T. Vu[1], Lars O.Svaasand[2]

[1]Beckman Laser Institute, University of California, Irvine 92715; [2]University of Trondheim, Norway

ABSTRACT

Frequency Domain Photon Migration (FDPM) non-invasively investigates the optical properties of multiple-scattering media such as tissue. In this work, FDPM is used to examine the optical properties of an EMT-6 murine tumor model in Balb/c mice. *In-Vivo* measurements are recorded before and after intravenous administration of the photosensitizer, chloroaluminum tetra-sulfonated phthalocyanine (CASPc). Optical properties are used to: 1) discriminate between tumor and healthy tissue and 2) monitor the rate of tumor drug uptake. Our data show substantial frequency-dependent contrast between tumor and normal tissue. In addition, drug pharmacokinetics are effectively recorded *in situ*. Although FDPM can quantitatively measure absorption and scattering coefficients in infinite homogeneous media, exact values of these optical properties are difficult to extract *in vivo* due to boundary condition uncertainties.

2. INTRODUCTION

In order to quantitatively study the optical properties of multiple-scattering media such as tissue, absorption must be distinguished from scattering. This can be accomplished using either time- or frequency-domain photon migration.[1,2] In Frequency Domain Photon Migration (FDPM), measurements are performed by launching amplitude-modulated laser light into tissue. Variations in the optical properties of the medium perturb the phase velocity (V_p) and amplitude of the diffusely propagating photon density waves.[3] Measuring density-wave phase and amplitude, with respect to the source, permits calculation of absorption and scattering coefficients.[4] Thus, frequency-domain techniques are real-time recordings which non-invasively report absorption (β) and effective scattering (σ_{eff}) coefficients *in situ*. These properties can be used in a variety of therapeutic and diagnostic techniques including: imaging tissue structure,[5] monitoring physiology,[6] and predicting optical dosimetry for laser-based procedures.[7]

In this work, FDPM is used to examine the optical properties of a live mouse tumor model. We first demonstrate that absorption and scattering coefficients of a tissue substitute (milk) spiked with the photosensitizing drug chloroaluminum sulfonated phthalocyanine (CASPc) can be accurately recorded using FDPM. Subsequent measurements are used to discriminate between tumor and healthy tissue on the basis of FDPM response. Finally, we show that CASPc pharmacokinetics can be recorded non-invasively in a living animal. Because of real-life boundary limitations, only relative phase velocity (V_p) measurements are reported for *in vivo* data.

0-8194-0792-5/92/$4.00

3. EXPERIMENTAL

3.1. Instrumentation

The Photon migration instrument is a modified multiharmonic Fourier transform phase and modulation fluorometer (SLM AMINCO, Urbana Ill, model 48000 MHF). Light is provided by an Argon-pumped dye laser with DCM dye operating at 650 nm. A Pockels cell, driven indirectly by the amplified output of a harmonic comb generator, is used to produce pulses with high harmonic content (multi-harmonic mode). These 5 MHz light pulses are transmitted to the sample by a 600 μm-core diameter optical fiber. Scattered light is collected by a second fiber placed some distance from the launch site. The frequency response between 5-250 MHz is recorded in a 16 second period. A complete description of the instrument has been provided elsewhere.

3.2. Methods

EMT-6 tumor cells (0.4 ml) are implanted subcutaneously in 8 - 12 week-old female Balb/c mice (0.25 kg). Tumors take about 7 days to reach optimal size, about 5 x 3 x 3 mm or 0.045 cm^3. These relatively small dimensions are required in order to ensure that measurements are not affected by tumor necrosis. Mice are shaved and a hair-removal ointment, Nair, is applied prior to measurement. Anesthesia is administered using a mixture of ketamine, xylazine and saline solution. Photon migration measurements are performed on tumor and healthy tissue before and after injection Measurements are recorded at different fiber distances and orientations. The pharmacokinetic time points are 0, 1, 3, 24, 44, 48 and 66 hours after injection of 2 mg/kg CASPc (Porphyrin Products, Logan UT).

4. RESULTS AND DISCUSSION

Figure 1, the calibration of CASPc in whole milk, illustrates that there is a linear relationship between the FDPM-calculated absorption coefficient, β, and the actual absorber concentration. All β values were calculated using previously reported expressions derived from diffusion theory.[4] As the concentration of CASPc increases, a commensurably higher absorption coefficient is measured, despite substantial scattering introduced by the milk solution. This study demonstrates that FDPM can detect small amounts of absorber in milk with accuracy and linearity, thus suggesting that FDPM is capable of similar performance in live tissues.

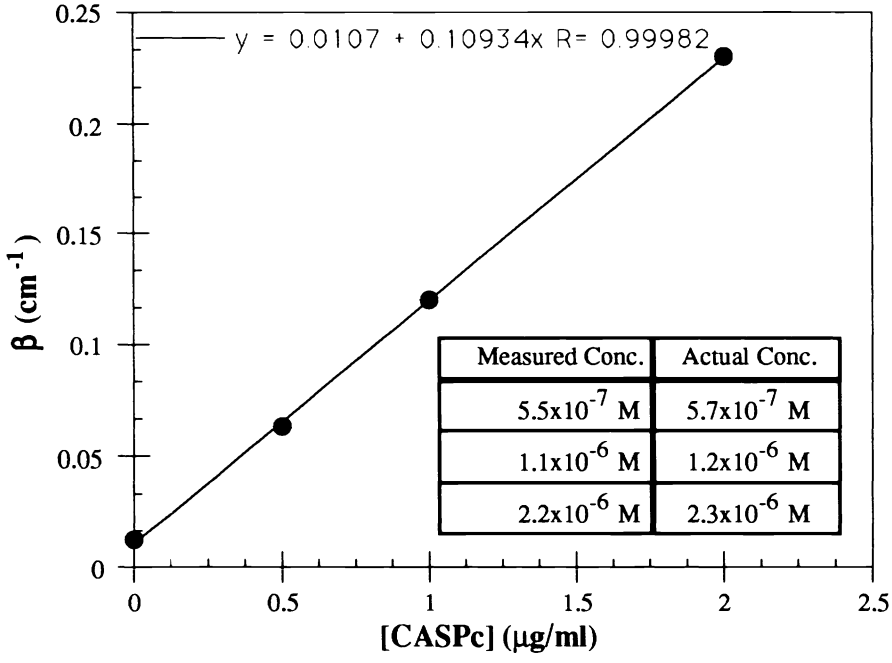

Figure 1. CASPc Calibration in whole (4%) milk

Figure 2 illustrates the phase response for normal and tumor tissues prior to CASPc injection (12 mm fiber separation). The Phase Slope differences (Phase Slope = $\Delta\phi$ / Δf where ϕ = phase lag in degrees and f = modulation frequency in MHz) clearly indicate that FDPM can discriminate between tissue type. Hence, FDPM-measured optical properties of tumor tissue differ from healthy tissue. The reduced phase slope observed for the tumor may be due to increased vascularity of the tumor. This would result in higher absorption and smaller phase shifts.[8] Of course, scattering properties may be different as well. The exact optical property values cannot be determined due to uncertain boundary conditions. These preliminary results also indicate that, in this case, single frequency measurements may not provide sufficient discrimination, unless conducted at high f. By measuring $\Delta\phi$ / Δf, however, relatively low frequencies can be employed to resolve tissue type.

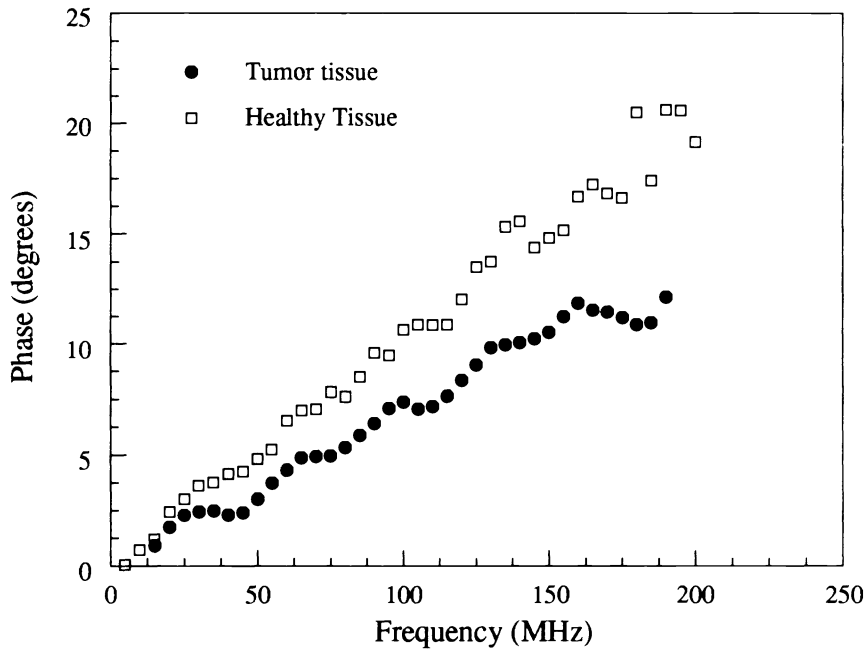

Figure 2. Phase shift vs. frequency for healthy and tumor tissue.

In figure 3a and 3b, phase is displayed as a function of modulation frequency, after CASPc injection, for 1 hour and 24 hour tumor measurements. Two different fiber orientations were investigated.

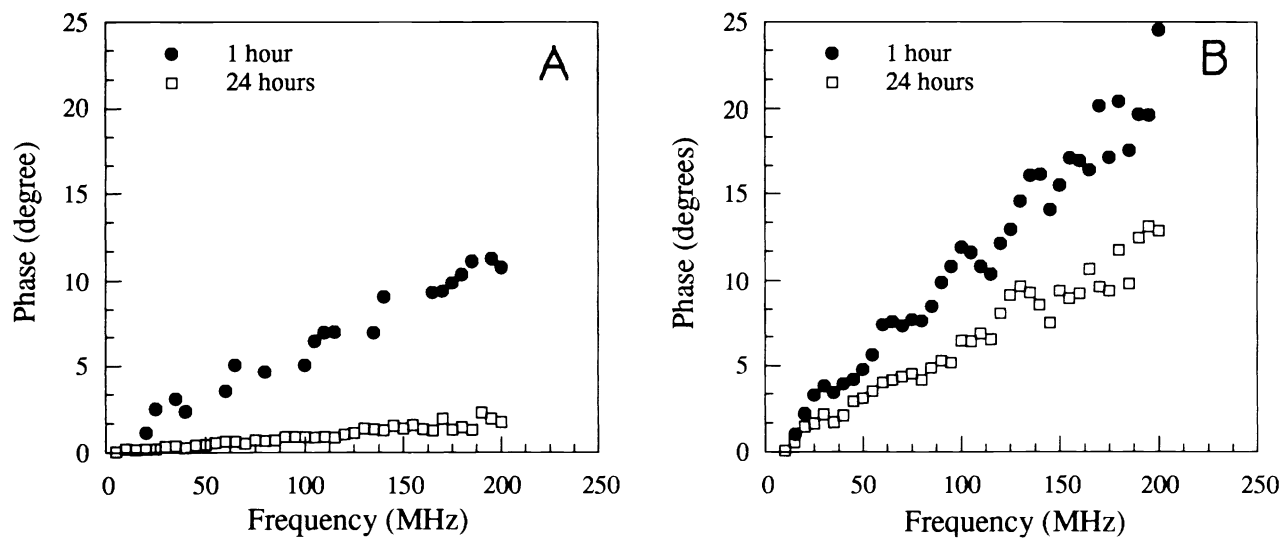

Figure 3. Tumor measurements 1 and 24 hours following intravenous administration of CASPc. (A) Phase as a function of frequency for 0.5 cm fiber separation; (B) Phase as a function of frequency for 1.2 cm fiber separation.

Figure 3a was measured with source and detector fibers separated by 0.5 cm at a 180° angle. In Figure 3b, fibers were separated by 1.2 cm and oriented at approximately 120°.

Clearly, the presence of CASPc substantially reduces the phase lag. This is expected, since the increased absorber concentration effectively captures light and diminishes the average time photons spend in the tissue. In addition, these data show that for a given range of frequencies, phase shift is highly sensitive to fiber optic orientation and distance. While the magnitude of the phase slope is clearly proportional to fiber separation, a greater phase slope difference between tumor and normal tissue is observed for the 5 mm case (Fig. 3a). This may be due, in part, to the fact that increased fiber separation and viewing angle (Fig. 3b) results in signal averaging over a larger tissue volume. Consequently, information from Fig. 3b is averaged over a viewing region which includes tumor and normal tissue. In contrast, Fig. 3a results are primarily indicative of tumor optical properties.

In Figure 4 relative phase velocity (V_p/c) is plotted as function of time after CASPc drug injection. Phase velocity is defined as:

$$V_p = 2\pi f / (\phi/d) \qquad (1)$$

where c is the velocity of light in the medium and d is the fiber separation distance. Since the absorption coefficient (β) is proportional to Vp^2, V_p/c is a sensitive indicator of absorption.[4] Assuming that tissue scattering properties are unchanged, the approximate 6-fold enhancement of V_p between hours 1 and 44 suggests that drug concentration increases by nearly a factor of 40 at the pharmacokinetic maximum.

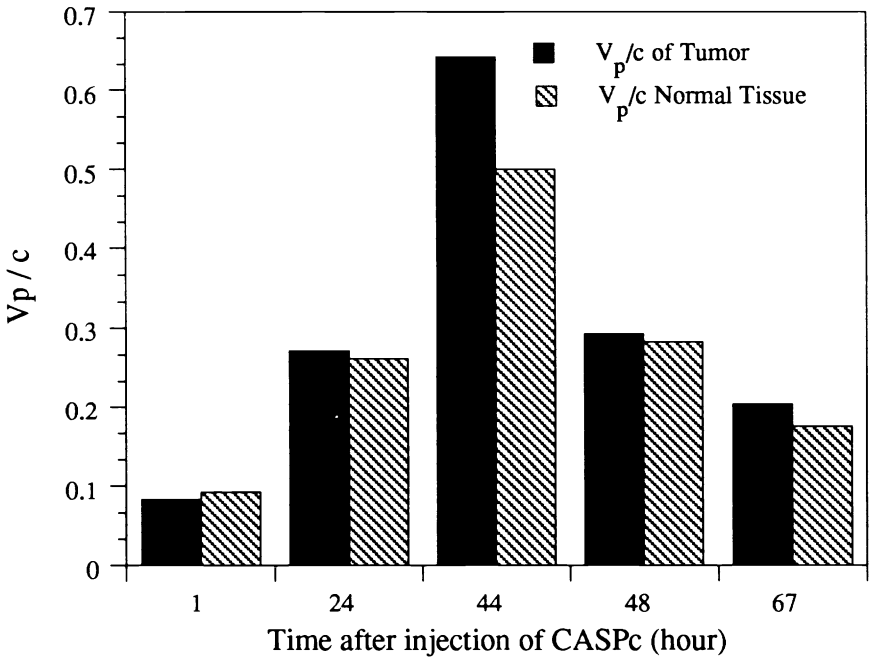

Figure 4. Relative phase velocity (V_p/c) vs. time after CASPc injection for tumor and normal tissue, 12 mm fiber separation.

Two conclusions can be drawn from this graph: first, the drug localizes at the tumor site. Comparison of V_p values at 44 hours yields (Tumor V_p)/(Normal tissue V_p) = 1.3. This suggests that CASPc concentration is almost twice as high in tumor vs. normal tissue. Secondly, the peak uptake of drug occurs 24 to 48 hours after injection. Both conclusions are in reasonable agreement with literature values for CASPc uptake and localization. Collectively, they demonstrate that FDPM can monitor the rate of drug uptake at targeted tissue sites.

Although these results do not provide the exact tumor concentration of CASPc, relative concentration is clearly and sensitively indicated by V_p/c values. We expect to overcome this limitation in future work by correlating these measurements to a more complete description of boundary and fiber geometry effects.

5. ACKNOWLEDGEMENTS

This work was performed with support from the University of California Cancer Research Coordinating Committee, the George Hewitt Foundation for Medical Research, and the Departement of Energy grant #DE-FG03-91ER61227.

6. REFERENCES

1. M. S. Patterson, B. Chance, B.C. Wilson, "Time-resolved reflectance and transmittance for the non-invasive measurement of tissue optical properties," Appl. Opt. 28, 2331-2336 (1989)

2. J. Fishkin, E. Gratton, M.J. VandeVen, W. W. Mantullin, "Diffusion of intensity modulated near-infrared light in turbid media," Proc. of SPIE, 1431, Los Angeles (1991).

[3] L. O. Svaasand, B.J.Tromberg, T-T. Tsay, R. C. Haskell, M. W.Berns, "On the Properties of Photon Density Waves in Tissues," Advances in Biological Heat and Mass Transfer, ASME 1991, HTD-Vol. 189/BED-Vol. 18, 85-89.

4. B. J. Tromberg, L. O. Svaasand, T-T. Tsay, R. C. Haskell, "Properties of photon density waves in multiple-scattering media," Appl. Opt., submitted.

5. E. M. Sevick, J. R. Lakowicz, H. Szmacinski, K. Nowaczyk, M. L. Johnson "Frequency-domain imaging of absorbers obscured by scattering, J. Photochem. Photobio., submitted.

6. E. M. Sevick, B. Chance, J. Leigh, S. Nioka, M. Maris, "Quantiation of time and frequency-resolved optical spectra for the determination of tissue oxygenation" Anal. Biochem. 195, 330-351 (1991).

7. S. L. Jacques, S. A. Prahl, "Modeling optical and thermal distributions in tissue during laser irradiation," Lasers in Surgery and Medicine 6, 494-503 (1987).

8. B. J. Tromberg, L. O. Svaasand, T. -T. Tsay, R. C. Haskell, M. W. Berns, "Optical property measurements in turbid media using frequency domain photon migration," Proc. SPIE 1525, Berlin, 52-58 (1991).