LA-UR-97- - 3 5 0 8

Approved for public release;
distribution is unlimited

Title: Optical Measurement of Drug Concentrations
in Tissue

Author(s): J. R. Maurant, CST-4
I. J. Bigio, CST-4
D. A. Jack, CST-4
T. M. Johnson, CST-4
H. D. Miller, CST-4

RECEIVED
OCT 01 1997
OSTI

Submitted to: DOE Office of Scientific and Technical Information (OSTI)

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

Los Alamos
NATIONAL LABORATORY

Los Alamos National Laboratory, an affirmative action/equal opportunity employer, is operated by the University of California for the U.S. Department of Energy under contract W-7405-ENG-36. By acceptance of this article, the publisher recognizes that the U.S. Government retains a nonexclusive, royalty-free license to publish or reproduce the published form of this contribution, or to allow others to do so, for U.S. Government purposes. Los Alamos National Laboratory requests that the publisher identify this article as work performed under the auspices of the U.S. Department of Energy. Los Alamos National Laboratory strongly supports academic freedom and a researcher's right to publish; as an institution, however, the Laboratory does not endorse the viewpoint of a publication or guarantee its technical correctness.

DISCLAIMER

**Portions of this document may be illegible
in electronic image products. Images are
produced from the best available original
document.**

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, make any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

Optical Measurement of Drug Concentrations in Tissue

Judith R. Mourant, Irving J. Bigio,* Darren A. Jack,
Tamara M. Johnson, and Heather D. Miller
Chemical Sciences and Technology Division

Abstract

This is the final report of a one-year, Laboratory Directed Research and Development (LDRD) project at Los Alamos National Laboratory (LANL). The aim of this project was to develop noninvasive fiber-optic methods for measuring drug concentrations in tissue. Such a system would make possible the study of chemotherapy drug kinetics at specific, targeted locations in the body after the drug is administered. The major result of this project is the development of techniques for measuring changes in absorption of a medium with unknown scattering properties. The developed method was verified by testing on several media with scattering properties in the range typically found for tissue.

Background and Research Objectives

The ability to noninvasively measure the concentrations of various drugs and compounds in tissues would provide a variety of medical benefits. A specific example of such a benefit is the case of chemotherapy drugs used for the treatment of various cancers. While the therapeutic benefit is determined by the tissue concentration of the drug in the targeted organ/tumor site, the one minimally invasive check available to the oncologist is to draw blood, assay the blood serum concentration and assume a relationship to the tissue concentration.¹ This assumption is often unreliable. More generally, the ability to track compound concentrations in tissue noninvasively would be a tremendous advantage in clinical pharmacology, especially for the development of new drugs.^{2,3} Another example is the case of photodynamic therapy, or PDT, a new treatment modality for cancer.⁴ For PDT, a photoactive compound with a selective affinity for cancerous tissue is administered topically, orally or intravenously. The molecule is then photoactivated with light of a specific wavelength, creating singlet oxygen and therefore damaging the tumor/malignancy. The ideal implementation of PDT requires delivery of the proper light "dosage," which in turn requires understanding the optical properties of the tissue and knowledge of the PDT drug concentrations present in the tissue to be treated.

* Principal Investigator, E-mail: ijb@lanl.gov

Importance to LANL's Science and Technology Base and National R&D Needs

Bioscience and biotechnology is one of the Laboratory's core competencies. Additionally, one of the aims of the Laboratory's tactical goals, "The Genome and Beyond," is to provide a basis for the development of a biotechnology/biomedical industry in New Mexico. The results of this project contribute to this goal, as is exemplified by the fact that we are applying for a patent on the technology.

Scientific Approach and Accomplishments

Measurements of absorption typically employ Beer's law, which is given in Eq. 1, where I_0 is the incident light intensity, I is the collected light, μ_a is the absorption coefficient, and L is the distance the light travels through the medium. With knowledge of L , the absorption coefficient can easily be measured. In a scattering medium, however, the light will not travel a straight path and L is generally not known.

$$I = I_0 e^{-\mu_a L} \quad (1)$$

Light entering a turbid medium from an optical fiber is quickly scattered in many directions. For a highly scattering medium some of the light will, after traveling through the medium, be reemitted on the same surface from which it entered. Fig. 1 shows an experimental setup for measuring the light emitted at specific distances from the source. The path length that the collected light travels through the medium will be much greater than the center-to-center separation of the source and detector fibers because of the many scattering events the light undergoes within the medium. The path length is expected to depend on the scattering properties of the medium. An underlying idea of this project is that there is an optimum fiber separation for which the dependence of the path length on the scattering parameters is smallest.

Highly scattering media, such as tissue, are typically characterized by an absorption coefficient, μ_a , a scattering coefficient, μ_s , and an anisotropy factor, g . The inverse of the absorption coefficient is the average distance a photon travels between absorption events. Depending on the wavelength, in tissue μ_a can vary from ~ 0 to 100 cm^{-1} . The inverse of

the scattering coefficient is the average distance a photon travels between scattering events. At each scattering event the photon's direction is changed by an angle θ . The expectation value of $\cos\theta$ is the anisotropy factor g . If most scattering events only change the photon's direction of travel by a small amount, then g is near 1. If scattering is isotropic, $g = 0$. A reduced scattering coefficient, $\mu_s' = \mu_s(1-g)$, can also be defined and is frequently used to characterize tissue. In tissue μ_s' typically has a value between 5 and 20 cm^{-1} , and g usually has a value between 0.8 and 0.98.

To investigate characteristics of light scattering at several separations between the light delivery and collection fibers, we performed Monte Carlo simulations using a previously described code.⁵ In Fig. 2 the average path length traveled by the collected photons is plotted against the scattering coefficient, μ_s , of the medium with the anisotropy factor, g , adjusted so as to keep μ_s' within the range 7.5 to 15 cm^{-1} . The delivery and collection fibers were separated by 1.75 mm. Fig. 2 shows that for this fiber separation the path length is nearly independent of the scattering properties. An interesting question is whether this essential insensitivity to scattering properties is valid for a range of absorption parameters. For the data in Fig. 2, $\mu_a = 0.5 \text{ cm}^{-1}$. More simulations were run with different values of μ_a and it was found that small ($\sim 0.1 \text{ cm}^{-1}$) changes in absorption did not affect the path length, but that larger changes in absorption alter the path length.

In order to verify the results of the Monte Carlo simulations, measurements were made of light transport through several highly scattering media with a variety of optical properties. As shown in Fig. 1, the light source was a xenon arc lamp and light was delivered to and collected from the scattering media by optical fibers. The light from the collection fibers was spectrally dispersed, and its intensity as a function of wavelength was measured by a CCD camera. We were able to simultaneously measure the light collected by fibers at separations of 0.75, 1.0, 1.48, 1.7, 1.95, and 2.2 mm from the delivery fiber using fiber optic probe #1 and at separations of 1.4, 1.86, 2.38, 2.94, 3.45, 4.1, and 4.5 mm using fiber optic probe #2.

Aqueous suspensions of polystyrene spheres and Intralipid-10% (a fat emulsion) were used as scattering media. Measurements were made in 5%, 10%, and 15% concentrations

of Intralipid-10%. These concentrations correspond to reduced scattering coefficients of 7 cm^{-1} , 15 cm^{-1} and 22 cm^{-1} at 550 nm with $g \sim 0.8\text{ cm}^{-1}$. The polystyrene spheres were $0.913\text{ }\mu\text{m}$ in diameter and were used in concentrations of 0.2%, 0.4%, 0.6% by weight.

This corresponds to reduced scattering coefficients of 7 cm^{-1} , 14 cm^{-1} and 21 cm^{-1} at 550 nm where $g = 0.920$. For absorbers, India ink and direct-blue dye #71 were used. India ink is a broad-band absorber in contrast to the blue dye which has an absorption band with a full-width-half-maximum of about 100 nm that peaks at 585 nm. The direct-blue dye was added to the scattering solutions in aliquots so as to change the absorbance each time by about 0.01 cm^{-1} at 585 nm. Spectra, i.e., the amount of light collected as a function of wavelength, were measured with no added absorber and after each addition of absorber.

The aim of the experimental measurements was to determine the dependence of the measured spectra on the amount of added absorber, and to show that for some specific fiber separation there is a property of the measured spectra that depends on the amount of added absorber, but not on the scattering properties of the medium. Data were analyzed by dividing each spectrum taken with blue dye present by the spectrum taken before the addition of any blue dye. The spectra were then normalized to 1 at 800 nm (a wavelength where the blue dye does not absorb), and the negative natural logarithm was taken over the entire wavelength range. These spectra will be referred to as "analyzed spectra" and examples are shown in Fig. 3. In Fig. 4 the areas under the traces of analyzed spectra from 579 to 591 nm are plotted versus the absorbance of the added blue dye at 585 nm for a source-fiber-to-detector-fiber separation of 1.48 mm. The different curves are for the 6 different scattering solutions. (India ink was not added to these scattering solutions.) Despite the large range of scattering properties in the different tissue phantoms, the curves in Fig. 4 are all very similar.

To determine the best choice of fiber separation, the percent difference in the areas from 579 to 591 nm of the analyzed spectra for the different scattering solutions was calculated by subtracting the maximum and minimum values of the area from 579 to 591 nm at an absorbance of 0.11 cm^{-1} and dividing by the minimum. [For example, from Fig. 4: $(1.85-1.52)/1.52 = 0.22$, and the percent difference is 22% at 1.48 mm.] This spectral-weight variation is plotted in Fig. 5, both for measurements made on tissue phantoms, for which there was no background absorption, and for measurements of tissue phantoms containing India ink and having a background absorption of $\sim 0.5\text{ cm}^{-1}$. For both sets of measurements it is found that the optimal fiber separation is about 1.5 mm.

The results of measurements made with different amounts of background absorber are shown in Fig. 6. Although all the data in Fig. 6 is for fiber center-to-center separations of 1.5 mm, the dependence of the "area of the analyzed spectra from 579 to 591 nm" on the amount of absorber added to the scattering media is different for the two cases of background absorbance. Therefore, some knowledge of the absorbance of the starting material is needed in order to quantitatively measure the amount of added absorber. Further analysis of the data has shown that the background absorbance needs to be known to an accuracy of about 0.2 cm^{-1} .

Therefore, the main results of this project are as follows: 1) Monte Carlo simulations suggest that at a fiber separation of 1.75 mm the path length of photons through a highly scattering medium does not depend on the scattering properties of the photons as long as the scattering properties are in the range typically found for tissue. 2) Experimentally, it was found that the optimal fiber separation is 1.5 mm. 3) A method of roughly estimating the absorbance of the starting media needs to be developed.

That the average path length is insensitive to the scattering properties is important for making measurements of small changes in absorption. Possible applications include concentration measurements of NADH (an important compound in cell metabolism), bilirubin (the yellow-orange pigment which accumulates in infants with jaundice), oxy- and deoxyhemoglobin, photodynamic therapy drugs, and chemotherapy drugs. For the case of chemotherapy drugs, we were unable to find published absorbance spectra. Therefore, we measured the absorbance of some chemotherapeutic compounds: doxorubicin, paraplalin, platinol, and taxol. All of the compounds had absorption bands in the UV and some had absorption bands in the visible. Optical in vivo measurement of the compounds with absorption bands only in the UV will be difficult because of the intrinsically strong absorption of tissue in this wavelength range. The measurement of concentrations of compounds that absorb in the visible is expected to be more feasible. Finally we note that the fact that the optimal fiber separation is only 1.5 mm allows for endoscopic implementations of the techniques developed in this LDRD project.

Publication

J. R. Mourant, I. J. Bigio, D. A. Jack, T. M. Johnson, and H. D. Miller, "Measuring absorbance in small volumes of highly scattering media: Geometries for which path lengths do not depend on scattering properties," Los Alamos National Laboratory document LA-UR-96-3671 (submitted to *Applied Optics*).

References

- [1] Pujol JL, Cupissol D, Gestinboyer C, Bres J, Serrou B, Michel FB, "Tumor-tissue and plasma concentrations of platinum during chemotherapy of non-small-cell lung cancer patients," *Cancer Chemotherapy and Pharmacology* **27**, 72-75 (1990).
- [2] Kerr DJ and Los G, "Pharmacokinetic principles of locoregional chemotherapy," *Cancer Surveys* **17**, 105-122 (1993).
- [3] Donelli MG, Zucchetti M, Dincalci M, "Do anticancer agents reach the tumor target in the human brain?" *Cancer Chemotherapy and Pharmacology* **30**, 251-260 (1992).
- [4] Schuitmaker JJ, Bass P, Vanleengoed HLLM, Vandermeulen FW, Star WM, Vanzandwijk N, "Photodynamic therapy: a promising new modality for the treatment of cancer," *J. of Photochem. and Photobiol. B* **34**, 3-12 (1996)
- [5] Mourant JR, Boyer J, Hielscher AH, Bigio IJ, "Influence of the scattering phase function on light transport measurements in turbid media performed with small source-detector separations," *Opt. Lett.* **21**, 546-548 (1996).

Figure 1. Schematic of the experimental setup.

Figure 2. The average path lengths of photons traveling from a delivery fiber to a collection fiber (in a backscatter geometry such as that shown in Fig. 1) as calculated by a Monte Carlo simulation. The source and detector fiber center-to-center separation was 1.75 mm and $\mu_a = 0.5 \text{ cm}^{-1}$.

Figure 3. Spectra taken after the addition of direct blue dye which have been divided by a spectrum with no blue dye, normalized to 1 at 800 nm, and then had the negative natural logarithm taken. (The source-detector separation was 1.48 mm. The scattering medium was 15% Intralipid-10%.) The values of μ_s are the absorbance of the scattering solution at 585 nm due to the addition of the direct blue dye.

Figure 4. Areas under the curve from 579 to 591 nm of analyzed spectra plotted versus the absorbance of the blue dye at 585 nm which was added to the scattering solutions for a source-detector separation of 1.48 mm.

Figure 5. Percent difference in the largest and smallest areas under the curve from 579 to 591 nm of the analyzed spectra for the 6 different scattering solutions plotted versus the source-detector fiber separation. Analyzed spectra were used for which the amount of added direct blue dye had an absorption of 0.11 cm^{-1} . The three scattering suspensions composed of 0.2%, 0.4% and 0.6% $0.913 \text{ }\mu\text{m}$ diameter spheres had reduced scattering coefficients of 6.3 cm^{-1} , 12.6 cm^{-1} and 25.2 cm^{-1} at 650 nm where $g=0.91$. The three scattering suspensions composed of 5%, 10%, and 15% Intralipid-10% had reduced scattering coefficients of 5 cm^{-1} , 10 cm^{-1} , and 16 cm^{-1} at 650 nm where $g=0.79$.

Figure 6. Areas under the curve from 579 to 591 nm of analyzed spectra plotted versus the absorbance at 585 nm of the blue dye which was added to the scattering solution for a fiber separation of 1.48 mm. Results are shown for the three scattering solutions composed of 0.2%, 0.4% and 0.6% by weight 0.913 μm diameter spheres with and without the addition of India ink.