

Measurements of Scattering and Absorption in Mammalian Cell Suspensions

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1. ABSTRACT

During the past several years a range of spectroscopies, including fluorescence and elastic-scatter spectroscopy, have been investigated for optically based detection of cancer and other tissue pathologies. Both elastic-scatter and fluorescence signals depend, in part, on scattering and absorption properties of the cells in the tissue. Therefore an understanding of the scattering and absorption properties of cells is a necessary prerequisite for understanding and developing these techniques. Cell suspensions provide a simple model with which to begin studying the absorption and scattering properties of cells. In this study we have made preliminary measurements of the scattering and absorption properties of suspensions of mouse mammary carcinoma cells (EMT6) over a broad wavelength range (380 nm to 800 nm).

2. INTRODUCTION

Optical techniques for determining and/or characterizing tissue pathologies measure spectroscopic properties of tissue. The development of such techniques necessitates understanding how various components of tissue (such as cells and vasculature) contribute to the optical signal. A small number of studies of the absorption and scattering properties of cell and organelle suspensions have already been made. Measurements of the absorption coefficient, μ_a , and the reduced scattering coefficient, μ_s' , have been made for yeast cells, hepatocyte cells, and isolated mitochondria at 780 nm.^{1,2} While these measurements are important and have yielded some insight into what the scattering centers may be under certain conditions, they are single wavelength measurements and have not addressed some of the issues that are important to fluorescence and elastic-scatter spectroscopy. Typically, for these techniques measurements are made over different and broader wavelength ranges. Particularly at the shorter wavelengths, it is possible that the primary scattering centers are smaller than at 780 nm. Also these techniques are expected to be sensitive to absorption of chromophores in the near-UV and visible. Therefore, we have made measurements of $\mu_a(\lambda)$, $\mu_s'(\lambda)$, the scattering coefficient, $\mu_s(\lambda)$, and the scattering anisotropy factor $g(\lambda)$ from <500 nm to 800nm for suspensions of mouse mammary carcinoma cells.

3. METHODS

3.1 Cell preparation

EMT6 mouse mammary carcinoma cells were cultured in α MEM medium containing 10% bovine calf serum, penicillin and streptomycin in a humidified incubator at 37° equilibrated with 5% CO₂ in 95% air (pH=7.4). Monolayer cultures were propagated in tissue culture flasks as described in detail elsewhere³ to maintain cell growth and viability. Cell suspensions for optical measurement were prepared by dissociating cells from the culture surface by treatment with 0.25% trypsin in a calcium- and magnesium-free buffer at pH 7.4 for 10 minutes at 37°. Excess ice-cold complete medium was then added, the mixture was mixed by repeated passage through a pipet, a sample was removed for counting, and the cell suspension was centrifuged (1500 rpm for 10 minutes). The cell pellet was then resuspended in ice-cold phosphate buffered saline at a concentration of $\sim 4.6 \times 10^7$ cells per ml and stored on ice until optical measurement. Cells were counted using an electronic particle counter equipped with a system for recording the volume distribution of the

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particles. Only those particles within the cell volume distribution were counted, and the mean volume of the cell population was estimated as the mean of the volume distribution of $>10^4$ cells³.

3.2 Measurement of the scattering coefficient

The scattering coefficient, μ_s , of the cell suspension was measured in a spectrophotometer. The transmittance of water was first measured and then the water was replaced by a dilute suspension of cells and the transmittance again measured. To insure that dilution was sufficient such that there was only one scattering or absorption event per pathlength of the cuvette, measurements were made at two dilutions and it was checked that the transmission was linear with cell concentration. Therefore, $\mu_t = \mu_s + \mu_a$ was measured. By subtracting off μ_a as measured below it was possible to determine μ_s .

3.3 Measurements of the absorption and reduced scattering coefficient

A schematic of the elastic-scatter measurement system is shown in Figure. 1. The light source is a continuous wave (cw) Xenon Arc lamp. Light is delivered to a spectrally flat diffuse reflecting reference material via a 200 micron (0.22 NA) optical fiber and delivered to the cell suspension via a 400 micron (0.22 NA) optical fiber. Light from the reference material is collected by a 200 micron (0.22 NA) optical fiber and light from the cell suspension is collected by a 600 micron (0.48 NA) fiber. Light from the collection fibers is incident onto separate sections of a thermoelectrically cooled CCD array (256x1024 element EEV CCD30). The signal from the reference was used to correct for any changes in the light output. Also, at the beginning or the end of data collection the cells and spheres are replaced by a spectrally flat diffuse reflecting material. This spectrum is used to correct for the wavelength dependence of the optical components of the system, such as the fiber transmission, grating efficiency and detector efficiency.

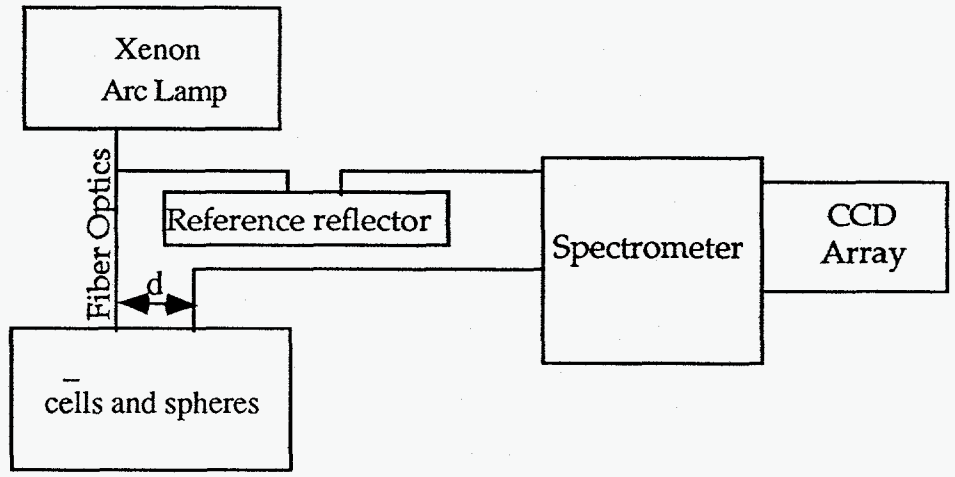


Figure 1. Schematic diagram of the optical measurement system.

The cell suspension was contained in a black container with a depth of 3 cm, a length of 2.5 cm, and a width of 1.5 cm surrounded by ice water. To assure that the cells and spheres did not settle the suspension was gently stirred during the measurements. For the measurements, the fibers were placed 1 to 2 mm into the suspension and the separation of the light delivery and collection fibers was varied. Derivations of equations for determining $\mu_a(\lambda)$ and $\mu_s'(\lambda)$ from measurements of frequency modulated light transport as a function of fiber delivery and collection separation in the diffusion approximation have been given by several authors.^{4,5} The application of these derivations to a cw system is fairly straightforward. In the diffusion approximation the photon spectral density, $U(\lambda)$, is given by:

$$U(\lambda) = \frac{S(\lambda)}{4\pi v D(\lambda) d} \exp \left[-d \left(\frac{\mu_a(\lambda)}{D(\lambda)} \right)^{1/2} \right] \quad (1)$$

where: v is the speed of the photons in the medium; d is the separation between the source and detector; $S(\lambda)$ is the source strength in photons per second; and

$$D(\lambda) = \frac{1}{3(\mu_a(\lambda) + \mu_s(\lambda))} \quad (2)$$

The detected elastic scatter signal, $I(\lambda)$, is proportional to the density of photons; therefore one can write:

$$I'(\lambda) = \frac{S'(\lambda)}{D(\lambda) d} \exp \left[-d \left(\frac{\mu_a(\lambda)}{D(\lambda)} \right)^{1/2} \right] \quad (3)$$

where the proportionality constants have been lumped together in the term $S'(\lambda)$. The wavelength dependence of $S'(\lambda)$ was determined by placing the light delivery and collection fibers over a material that reflects all wavelengths equally and measuring the detected signal, $f(\lambda)$. If $c = S'(\lambda)/f(\lambda)$ and $I(\lambda) = I'(\lambda)/f(\lambda)$, then $S'(\lambda)$ can be replaced by c in equation 3, if $I'(\lambda)$ is replaced by $I(\lambda)$.

The natural logarithm of $I(\lambda, d)$ is a straight line as a function of fiber separation, d :

$$\ln(I(\lambda) d) = \ln\left(\frac{c}{D(\lambda)}\right) - d \left(\frac{\mu_a(\lambda)}{D(\lambda)} \right)^{1/2} \quad (4)$$

The slope (m) and intercept (b) of $\ln(I(\lambda, d)$ versus d are:

$$m(\lambda) = - \left(\frac{\mu_a(\lambda)}{D(\lambda)} \right)^{1/2} \quad \text{and} \quad b(\lambda) = \ln\left(\frac{c}{D(\lambda)}\right) \quad (5,6)$$

Therefore:

$$\mu_a(\lambda) = \frac{c m^2(\lambda)}{e^{b(\lambda)}} \quad \text{and} \quad \mu_s'(\lambda) = \frac{m^2(\lambda)}{3\mu_a(\lambda)} - \mu_a(\lambda) \quad (7,8)$$

To solve for μ_a and μ_s' it is necessary to determine c . This was done by measuring the elastic scatter signal as a function of fiber separation before and after the addition of a known amount of CoSO_4 . By comparing the two absorption curves it is possible to determine c .

The scattering and absorption coefficients of the spheres themselves are small enough that edge effects are a problem for the small volumes of cells which are typically available for these measurements. Beauvoit et al. have solved this problem by immersing the cells in a media with similar absorption and scattering coefficients.^{1,2} While this method works well for measurements at a single wavelength, it is not easily applicable to measurements over a broad wavelength range.

An alternative solution is to add polystyrene spheres to the cell suspension in order to make the scattering density high enough as to alleviate any problems with edge effects. Measurements were made both of the cell suspension alone and of the cell suspension after the addition of polystyrene spheres. Polystyrene spheres with a diameter of 0.895 microns were added in 4 increments of 300 μl with measurements of the suspension made after each addition of spheres.

The calculation of μ_s' however is not straight forward. Although μ_s is linearly additive, μ_s' is not:

$$\mu_s'(cells\&spheres) - \mu_s'(spheres) = \mu_s'(cells) + \text{error} \quad (9)$$

In this case:

$$\text{error} = \mu_s(cells)*(g_c - g_{sc}) + \mu_s(spheres)*(g_s - g_{sc}) \quad (10)$$

where $g_c = \langle \cos\theta \rangle$ for the cells alone, $g_s = \langle \cos\theta \rangle$ for the spheres alone, and g_{sc} is the value of $\langle \cos\theta \rangle$ for a suspension of cells and spheres. After some algebra an expression for g_{sc} can be obtained in terms of known scattering parameters of the added spheres, the measured scattering coefficient of the cells and the measured reduced scattering coefficient of the suspension of cells and spheres. From measurements of g_{sc} for two different combinations of cells and spheres it is possible to determine g_c .

4. RESULTS

The results of the measurements of $\mu_s(\lambda)$ for the suspension of EMT6 cells on a spectrophotometer are shown in Figure 2. The scattering coefficient for a concentration of 4.6×10^7 cells/ml is plotted from 350 to 850 nm. Over this wavelength range μ_s decreases slowly with wavelength.

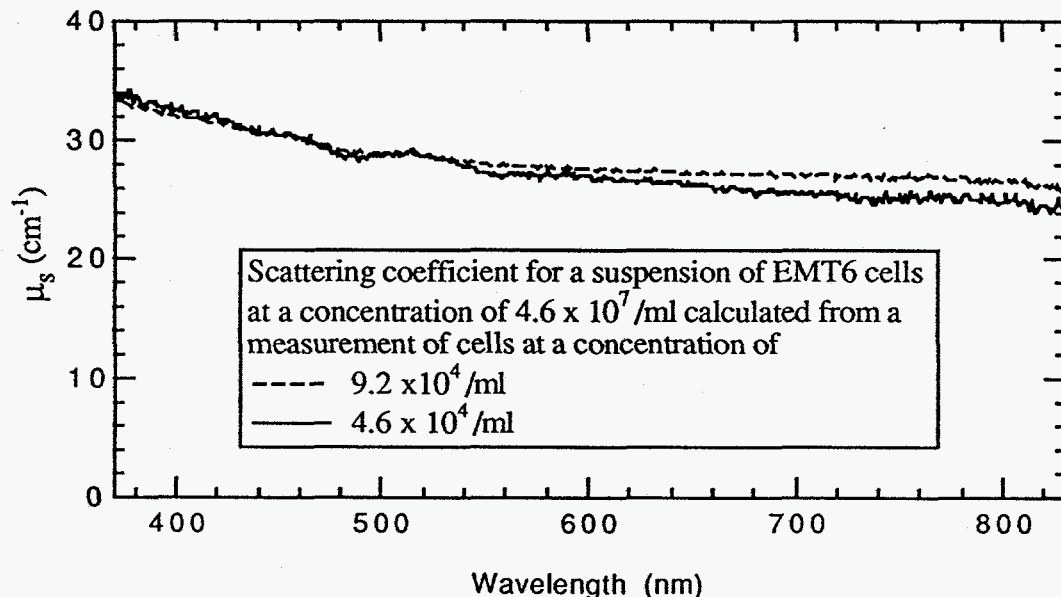


Figure 2. The total extinction coefficient, $\mu_a + \mu_s$ as a function of wavelength for a suspension of EMT6 cells at a concentration of 4.6×10^7 cells per ml.

Elastic-scatter signals, $I(\lambda, d)$, were fit to straight lines from $d=0.8$ cm to $d=1.12$ cm and from $d=1.0$ cm to $d=1.25$ cm. As described above, the absorption coefficient, $\mu_a(\lambda)$, was then calculated for each set of fits. The results for two combination of cells and spheres are shown in

Figure 3. The only significant differences in the curves are below 480 nm where the data calculated for fiber separations of 0.8 to 1.12 cm is different from the data calculated for fiber separations of 1 to 1.25 cm. This is an indication that the diffusion approximation is not valid at these wavelengths, presumably because of the large absorbance. Nonetheless the data below 480 nm is valuable. There is clearly a peak in the absorbance at 410 nm and the absorption coefficient has a wavelength dependence typical of heme compounds. Since there is no hemoglobin present, the most likely candidate is a cytochrome.

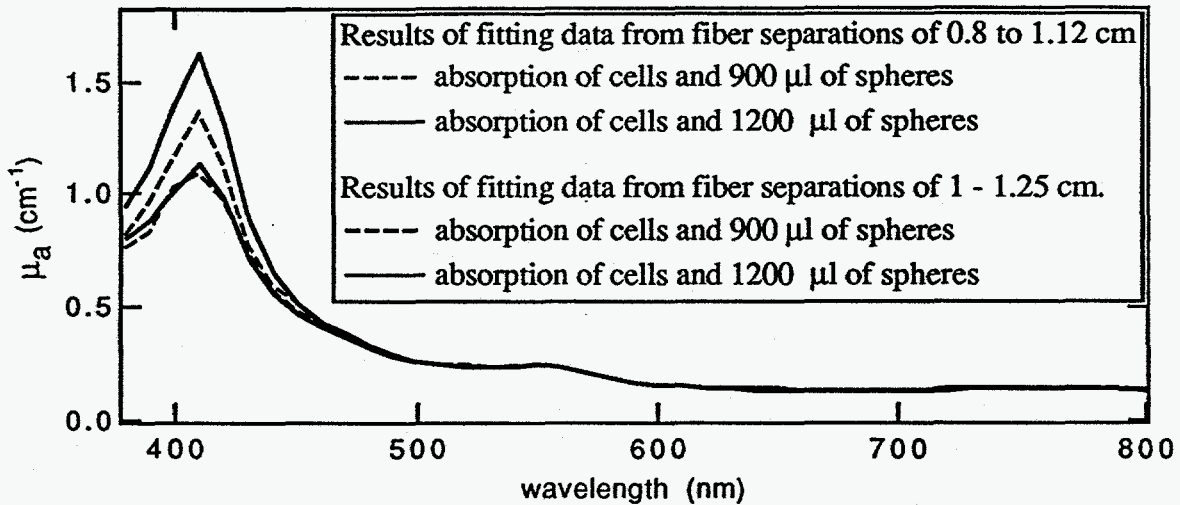


Figure 3. Measurements of the absorption coefficient as a function of wavelength for suspensions of EMT6 cells at a concentration of 4.6×10^7 cells per ml.

Figure 4 shows the value of g obtained by fitting the data from fiber separations of 1-1.25 cm. Data is only plotted for wavelengths greater than 500 nm, because as evidenced by Figure 2, at lower wavelengths, the diffusion approximation did not hold. The value is in the range expected for tissue. At this point these data are quite preliminary. Although error bars have not been formally calculated, the error in g is estimated to be ± 0.4 . Figure 5 shows a calculation of μ_s' based on the figures 2 and 4. Again the errors, although not formally calculated, are large and are estimated to be on the order of $\pm 1.5 \text{ cm}^{-1}$.

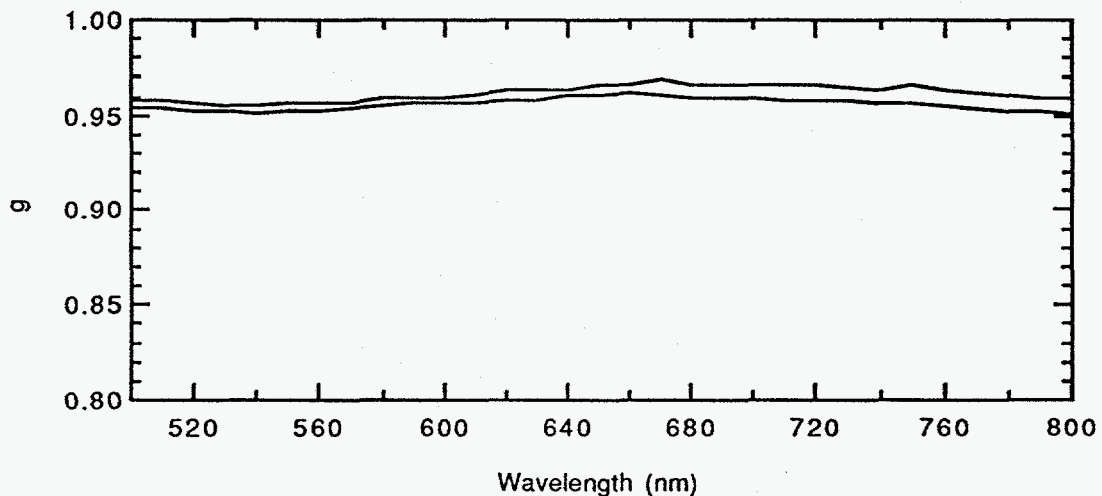


Figure 4. Preliminary estimates of the expectation value of the cosine of the scattering angle (g) as a function of wavelength for an EMT6 cell.

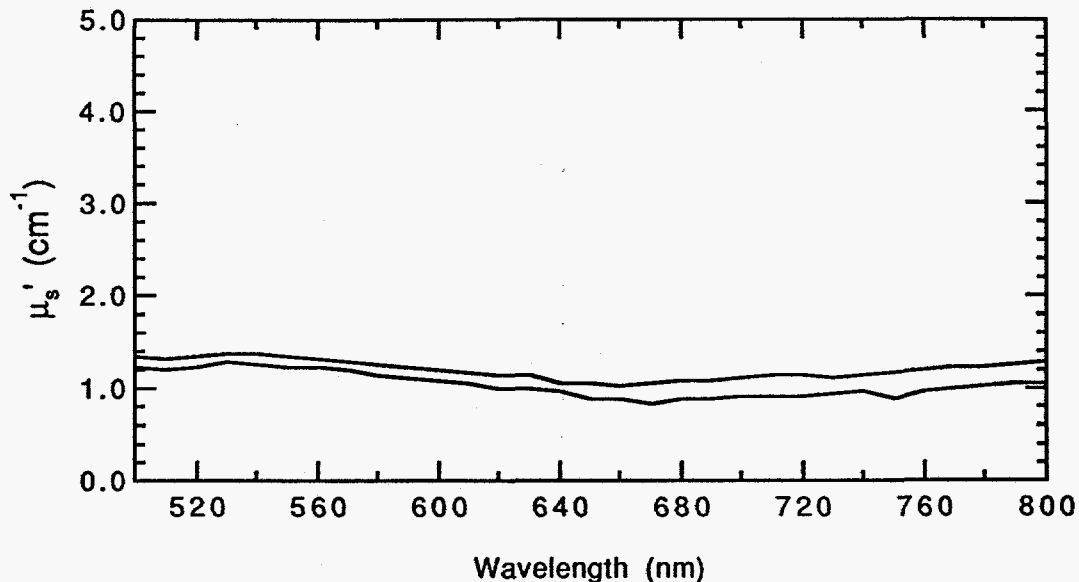


Figure 5. Preliminary estimates of the reduced scattering coefficient as a function of wavelength for a suspension of EMT6 cells at a concentration of 4.6×10^7 cells per ml.

5. DISCUSSION

Measurements of $\mu_a(\lambda)$, $\mu_s'(\lambda)$, $\mu_s(\lambda)$, and $g(\lambda)$ have been made over broad wavelength ranges. The results for $\mu_a(\lambda)$ showed that the presence of a heme compound (presumably a cytochrome) could easily be measured. Although, the values for $\mu_s'(\lambda)$ and g are still preliminary they are in the expected range. In tissues, g typically has a value between 0.8 and 1.0. For Baker's yeast Beauvoit et al.¹ obtained $\mu_s' = 3 \text{ cm}^{-1}$ at a concentration of 7.2×10^8 cells per ml. Our value of 1 cm^{-1} for EMT6 cells at a concentration of 4.7×10^7 cells per ml gives roughly 1/3 the amount of scattering per cell volume given that the volume of the Yeast cells was $83 \pm 12 \mu\text{m}^3$ and the volume of the EMT6 cells was $1.58 \pm 0.32 \times 10^3 \mu\text{m}^3$.

6. REFERENCES

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