OPTICAL PROPERTIES OF BRAIN TISSUE

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(Paper JBO-029 received July 31, 1995; revised manuscript received Nov. 16, 1995; accepted for publication Nov. 23, 1995)

ABSTRACT

The single scattering properties (extinction coefficient and scattering function at small forward scattering angles) of bovine and swine brain were obtained from transmissometric measurements on thin slices of tissues at λ =633 nm. Large differences between the optical properties of white and gray matter were observed, whereas minor differences were found between bovine and swine samples. For gray matter, values between 10 and 30 mm⁻¹ were obtained for the extinction coefficient. The scattering function was strongly forward peaked, indicating a highly anisotropic scattering. For white matter, values between 100 and 250 mm⁻¹ were measured for the extinction coefficient. These values were so large that it was impossible to obtain a sample thin enough to measure the scattering function in single scattering conditions.

Key Words brain optical properties; extinction coefficient; scattering function.

1 INTRODUCTION

A knowledge of the optical properties of biological tissues is important for constructing models describing photon migration. These models are useful for many purposes: for example, to properly use photodynamic therapy, to study imaging techniques, or to interpret spectroscopic measurements. An important diagnostic application is the monitoring of blood oxygenation in the brain to prevent damage from low arterial blood oxygenation and abnormal cerebral blood flow. For these applications it is necessary to know the optical properties of the brain in order to develop models for light distribution and to interpret the diagnostic data. In spite of much progress in tissue optics, there is a lack of information on optical properties of tissues. This fact appears from the data reported in a recent review on optical parameters of tissues.¹ A large spread of values for the different parameters appears in these data. The large discrepancies among the results reported by different authors cannot be explained solely by the variability of optical parameters intrinsic to different types of tissues. As discussed in Ref. 1, the discrepancies are probably due, at least in part, to different methodologies used for measurements. Other discrepancies can arise from the method of tissue preparation.

Biological tissues are highly scattering media. Typical values for the scattering coefficient are between 10 and 100 mm⁻¹, corresponding to meanfree-paths between two subsequent scattering events in the range 100 to 10 μ m. Thus, photons migrating through the tissue undergo very frequent scattering events. Biological tissues exhibit a scat-

Address all correspondence to Giovanni Zaccanti, Università di Firenze, Dipartimento di Fisica, Via Santa Marta 3, Firenze 50139, Italy. E-mail: zaccanti@dffs.unifi.it tering function $p(\vartheta)$ having a pronounced peak in the forward direction. The scattering function is a quantity that is proportional to the distribution of scattered power. Large values for the average of the cosine of the scattering angle ϑ (the asymmetry factor *g*) are expected for biological tissue.

In this paper we report the results of measurements of single scattering properties of brain samples at λ =633 nm. These measurements (scattering coefficient and scattering function for small scattering angles) were carried out on thin slices of brain of pigs and cows within 2-5 hours postmortem. Measurements on brain samples showed significant differences between the optical properties of gray and white matter. Minor differences were measured between swine and bovine samples. Values for the extinction coefficient were between 13 and 29 mm^{-1} for gray matter and between 133 and 228 mm⁻¹ for white matter. A scattering function highly peaked in the forward direction was especially found for gray matter, indicating a highly anisotropic scattering and an asymmetry factor probably close to 0.99.

2 METHODOLOGY FOR MEASURING THE EXTINCTION COEFFICIENT AND ESTIMATION OF ERROR DUE TO MULTIPLE SCATTERING

The extinction coefficient μ_e describes the attenuation of a light beam propagating through a turbid medium. A collimated light beam (emitted power P_e) propagating through a slice of homogeneous diffusers with thickness *L* is attenuated by both the absorption and the scattering effects of the turbid medium. The attenuated collimated beam (power

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 P_0) emerging from the slice is related to the extinction coefficient by the Lambert–Beer law:

$$P_o = P_e \exp(-\mu_e L). \tag{1}$$

Measuring P_e and P_o , the extinction optical thickness τ_e can be obtained:

$$\tau_e = \ln \frac{P_e}{P_o}$$
 and $\mu_e = \frac{\tau_e}{L}$. (2)

The extinction coefficient can be written as $\mu_e = \mu_a + \mu_s$, where μ_a and μ_s are respectively the absorption and the scattering coefficient.

The determination of P_o is difficult when measurements are carried out on turbid media having $\mu_s \gg \mu_a$, as is the case for biological tissues at visible or near-infrared (NIR) wavelengths. Due to the scattered power P_s that unavoidably reaches the photometer, a quantity $P = P_o + P_s$ is measured instead of P_o . The value of the optical thickness τ_m obtained by using in the Lambert-Beer law the measured quantity $P_o + P_s$ instead of P_o , i.e., $\tau_m = \ln[P_e/(P_o + P_s)]$, is thus underestimated. The quantity P_s depends on both the characteristics of the optical receiver (area, angular field of view) and the scattering properties of the diffusing medium. When measurements are carried out on diffusing media having a highly forward-peaked scattering function, the error on μ_e due to the scattered received power can be very large even for measurements carried out at $\tau_e < 1$. In a previous paper² dealing with the error on μ_e due to scattered received power, we showed an error as large as 50% when measurements were carried out on large polystyrene spheres by using an optical receiver having a field of view α =3 degrees even when measurements were carried out with τ_e approaching zero.

Since for biological tissues a scattering function having a highly forward peak is expected, measurements should be carried out by using a receiver having a small angular field of view. With the experimental setup we used, having α =0.072 degrees, the error due to scattered received power is expected to be insignificant when measurements are carried out at small values of τ_e , even when they are carried out on a medium having a very forward peaked scattering function: at $\tau_e=1$ the fraction of single scattered received power remains smaller than $P_o/100$ (and thus the error on τ_e is smaller than 1%) even if $p(\vartheta=0)$ is as large as 1000. As long as measurements are carried out at optical thicknesses for which $P_s \ll P_o$, by increasing the geometrical thickness of the slice we expect that the measured optical thickness $\ln(P_e/P)$ linearly increases with a slope given by the extinction coefficient.

To minimize the error on μ_e , it is necessary to minimize the quantity of scattered received power. This can be done by illuminating the sample with a collimated light beam and measuring the transmit-



Fig. 1 Limits due to multiple scattering on the determination of the extinction coefficient. The curve refers to attenuation measurements carried out on a highly packed diffusing medium (water and polystyrene spheres Φ =15.8 μ m, concentration 20%). In this case the optical depth versus the geometrical thickness is a straight line, with the angular coefficient equal to μ_e , for τ_m smaller than about 11.

tance with a receiver having a small angular field of view α : only photons emerging from the diffusing medium with angles smaller than α can reach the detector. However, when τ_e increases, the ratio P_s/P_o is expected to increase, and for large values of τ_e , P_o is expected to become a small fraction of P_s : under these conditions transmissometric measurements give wrong results. We denote with $au_{
m elim}$ the value of τ_e for which $P_s = P_o$. The corresponding error due to scattered received power results: $\tau_m - \tau_{\text{elim}} = -\ln 2 \approx -0.7$. Since for $\tau_e > \tau_{\text{elim}}$ the error is expected to increase quickly, measurements of μ_e should be carried out at $\tau_e < \tau_{\text{elim}}$. A rough evaluation of $\tau_{\rm elim}$ can be obtained by making some simplifying assumptions. If we assume: (1) a nonabsorbing medium; (2) $P_o \ll P_e$; (3) due to many orders total of scattering the scattered power $P_{st}(P_{st}=P_e-P_e)$, is isotropically remitted, remaining localized near the light beam (in our measurements the thickness L of the slice was very small compared with the diameter φ of the light beam); then the illuminated sample acts as an isotropic source and the received scattered power is the fraction emitted within the small solid angle subtended by α :

$$P_s(\alpha) \cong P_e \frac{\alpha^2}{4} \,. \tag{3}$$

The angular field of view of our experimental setup was α =0.072 degrees and the corresponding value of τ_{elim} obtained by using Eq. (3) to evaluate P_s is $\tau_{\text{elim}}\cong$ 14.7. In spite of the very rough approximation on the angular distribution of the scattered power, this value accounts for the experimental results as shown in Fig. 1, where attenuation measurements on polystyrene spheres (diameter 15.8 μ m, concentration 20%) are reported. The optical thickness versus the geometrical thickness is a straight line for τ_m smaller than about 11. As *L* increases, the slope

of the experimental curve decreases and the optical thickness is slightly dependent on geometrical thickness (the diffusion regime).

3 METHODOLOGY FOR MEASURING THE SCATTERING FUNCTION AT SMALL SCATTERING ANGLES

The scattering function $p(\vartheta)$ represents the probability for a photon to be scattered, per unit solid angle, of an angle ϑ from the original direction of motion. The scattering function was assumed normalized to 1 on the whole solid angle. $p(\vartheta)$ is usually measured by goniometric measurements: the sample of diffusing material is illuminated with a collimated light beam and the light scattered at different angles is measured with a photometer. If the optical thickness of the medium is small enough, multiple scattering effects can be disregarded and the measured power is simply related to the scattering function. To avoid multiple scattering effects, it is necessary to use samples with a thickness smaller than the mean-free-path between two subsequent scattering events, i.e., $\tau_s < 1$, with $\tau_s = \mu_s L$, the optical thickness only due to scattering. If $\tau_s < 1$, the probability that a photon will undergo more than one scattering event crossing the slices is small. With goniometric measurements it is difficult to separate the scattered light (generally a small fraction) from the unscattered component when measurements are carried out at small scattering angles.^{3,4} Goniometric measurements of $p(\vartheta)$ are often reported only for angles larger than ≈ 2 degrees. The results referring to small angles are often obtained by using an extrapolation procedure.³ Since for biological tissues $\approx 50\%$ of the scattered power is expected to be emitted within a narrow forward lobe, measurements at small scattering angles can give information comparable to that obtained from the remaining angular range. For this reason we focused our attention on the scattering function at small scattering angles.

The value of $p(\vartheta)$ at small angles was obtained from the repetition of transmissometric measurements by using different values for the angular field of view α of the receiver. By using a light beam with a small divergence (divergence smaller than the smallest value of α used) and carrying out measurements in the single scattering regime (τ_s <1), the mean value p_{ij} of the scattering function in the angular range α_i , α_i can be obtained as⁵

$$p_{ij} = \frac{1}{2\pi \left(\cos\frac{\alpha_i}{n} - \cos\frac{\alpha_j}{n}\right)} \frac{P(\alpha_j) - P(\alpha_i)}{a\tau_e P_o}, \quad (4)$$

where $a = \mu_s / \mu_e$ is the single scattering albedo and n is the refractive index of the medium surrounding the diffusers. Our measurements were carried out at λ =633 nm at which $\mu_a \ll \mu_s$ is expected, so we assumed a=1. The value n=1.40 was assumed for



Fig. 2 Scheme of the experimental setup. The light beam was an He-Ne laser. The diaphragm D with radius R was placed in the focal plane of the lens in front of the detector.

the biological tissue.⁶ This procedure has been successfully used for measurements on both suspensions of calibrated microspheres and on natural fogs.⁵

4 EXPERIMENTAL SETUP

Both the extinction coefficient and the phase function were obtained from transmissometric measurements. The scheme for both sets of apparatus is shown in Fig. 2. The tissue was illuminated by a light beam having a diameter φ =3 mm and a divergence ≈ 0.017 degrees obtained with an He-Ne laser and a beam expander. Calibrated neutral density filters were used to avoid saturation effects when measurements were taken at small optical depth. The diaphragm D1 was used to limit the contribution of multiple scattering. The diaphragm D (radius R), placed in the focal plane of the lens in front of the detector, determines the angular field of view of the receiver. Two different optical receivers were used for measurements of μ_e and $p(\vartheta)$. For measurements of the extinction coefficient, the focal length was 160 mm and the diaphragm D was a small pinhole (R=0.2 mm): the field of view was α =0.072 degrees. This value for α , which is slightly larger than the divergence of the laser beam, has been chosen to obtain an easy and noncritical alignment. The detector was a photomultiplier and the signal was measured by using a lock-in amplifier.

With the methodology used to determine $p(\vartheta)$, transmissometric measurements should be repeated for several values of the angular field of view. The optical receiver used was basically similar to the one fully described in Ref. 5. The focal length was 83 mm and different values of α_i were obtained by using a variable diaphragm. Measurements were repeated for eight values of *R* ranging from 0.4 to 5.5 mm and corresponding to values of the receiver angular field of view ranging from 0.29 to 3.8 degrees.

5 TEST OF MEASUREMENT METHOD FOR THE SCATTERING FUNCTION AND ERROR DUE TO MULTIPLE SCATTERING

The measuring procedure for $p(\vartheta)$ described above was tested using different suspensions of calibrated



Fig. 3 Tests of the measuring method for $p(\vartheta)$. The figure shows a comparison between experimental results obtained from measurements carried out on polystyrene spheres and the results of Mie theory (continuous lines). Two different sizes have been used: $\Phi=11.9 \ \mu m$ (solid symbols) and $\Phi=25 \ \mu m$ (hollow symbols). The optical depths were 0.38 and 0.7, respectively.

polystyrene microspheres in twice distilled water. The results obtained were compared with Mie theory. The mean diameter (Φ) and the standard deviation (SD) of the polystyrene spheres used were: Φ =11.9 μ m, SD=1.9 μ m; Φ =25 μ m, SD=5.8 μ m. These sizes have been chosen because their scattering functions at small angles present a highly forward peak, as expected for biological tissues.

To avoid multiple scattering error, it is necessary to carry out measurements at $\tau_s < 1$. The scattering cell was 4 cm thick. A stirrer was used to ensure the homogeneity of the suspension. The experimental results obtained are shown in Fig. 3 (symbols). The figure refers to spheres of 11.9 μ m and 25 μ m having respectively an optical thickness τ_m equal to 0.38 and 0.7. In the figure the results of Mie theory are also shown (a Gaussian size distribution was assumed). Taking into account the experimental errors, the agreement between theory and measurements is good for all types of spheres.

To evaluate the error on $p(\vartheta)$ due to multiple scattering when measurements are carried out for large values of τ_s , measurements were repeated for different values of the optical thickness. Since in our measurements $\mu_a \ll \mu_s$, large values of τ_e mean also large values of τ_s . Figure 4 shows the experimental results obtained for spheres with Φ =11.9 μ m and τ_m >1. The differences between the results obtained for different values of τ_m are due to multiple scattering: if τ_m is increased, the value of $p(\vartheta)$ is overestimated and its profile becomes more "flat" since multiple scattering effects produce a more isotropic angular distribution with respect to the single scattering function. For example, when τ_m =3.7, the measured value of $p(\vartheta)$ is overestimated by a factor of 1.7 at ϑ =0.2 degrees and by a factor of 12 at ϑ =2.6 degrees. These results can be useful in estimating the scattering function when it is impossible to carry out measurements in the single scattering regime.



Fig. 4 Effect of multiple scattering on measured scattering functions for polystyrene spheres with Φ =11.9 μ m. Symbols refer to different optical depths: from lower to upper: τ_m =0.38, 1, 1.3, 1.6, 1.9, 2.6, 3.7, and 4.7, respectively. The solid line is calculated from Mie theory.

6 SAMPLE PREPARATION

In order to prepare thin slices of brain, the procedure described in Ref. 4 was used: a small piece of tissue was taken and directly squeezed between two optical glasses separated by a spacer without the need to freeze and slice the tissue. A drop of saline was used to improve the optical contact between the tissue and glass in order to prevent scattering from rough surfaces. The actual thickness of the sample was measured with a micrometer. Brain samples were obtained from cows (18 to 20 months) old) and pigs (10 to 12 months old). Measurements were carried out at room temperature (20 to 24°C) within 2 to 5 hours postmortem. Note that the results obtained may not necessarily provide an accurate representation of in vivo brain optical properties due to variations of the scattering properties that can occur postmortem or during sample preparation.

7 EXPERIMENTAL RESULTS

7.1 EXTINCTION COEFFICIENT

Figure 5 shows two examples of attenuation measurements carried out on samples of gray and white matter of swine [Fig. 5(a)] and bovine brain [Fig. 5(b)]. The figure shows the attenuation $[\ln (P_e/P)]$ measured for different thicknesses of the sample. Each value was obtained as the mean of eight measurements on different portions of the sample. The extinction coefficient was obtained from the slope of the straight line best fitting the experimental results. To avoid errors due to scattered received power, only measurements corresponding to $\tau_m \leq 10$ were used for the fitting procedure. The straight lines representing the best fit for white and gray matter are also reported.

The figure shows the large difference between the extinction coefficients for white and gray matter. Similar results were obtained for other samples used. Minor differences were observed between



Fig. 5 Examples of attenuation measurements carried out on samples of gray and white matter of swine (a) and bovine brain (b). Symbols are experimental results; straight lines are best fits.

swine and bovine samples. Table 1 summarizes the results obtained from measurements carried out on three samples of swine and four samples of bovine brain. The mean value of μ_e was 21 mm⁻¹ for gray matter and 188 mm⁻¹ for white matter. The corresponding mean-free-paths are \approx 50 μ m and \approx 5 μ m respectively.

7.2 SCATTERING FUNCTION AT SMALL SCATTERING ANGLES

To avoid errors due to multiple scattering, measurements of scattering functions should be carried out

Table 1Summary of the results obtained from measurements car-ried out on three samples of swine and four samples of bovinebrain at 632.8 nm.

| Extinction coefficient (mm ⁻¹) | | | | |
|--|-------------|--------|--------------|--------|
| | Gray matter | | White matter | |
| Sample No. | swine | bovine | swine | bovine |
| 1 | 16.1 | 23.6 | 195 | 166 |
| 2 | 13.1 | 21.6 | 133 | 220 |
| 3 | 18.5 | 27.9 | - | - |
| 4 | | 28.7 | | 228 |



Fig. 6 Scattering functions for gray matter measured on (a) three samples of swine and (b) on four samples of bovine brain. The optical thickness for the swine samples was 0.4 for sample 1, 0.6 for sample 2, and 0.7 for sample 3. The optical thickness for the bovine samples was 0.85, 0.75, 0.7, and 0.8 for samples 1, 2, 3, and 4 respectively.

in the single scattering regime on samples with an optical thickness of less than about 1. It was possible to obtain samples with a sufficiently small optical thickness only for gray matter. The results obtained for white matter are thus strongly affected by multiple scattering: this involves an overestimation of the scattering function. Experimental results for $p(\vartheta)$ are shown in Fig. 6 for gray matter and in Fig. 7 for white matter. Each value was obtained as the mean of four measurements on different portions of the sample. Figures 6(a) and 7(a) are for swine, whereas Figs. 6(b) and 7(b) are for bovine brain. The different marks indicate the different samples. The figures show a highly forward-peaked scattering function, especially for gray matter. More than 50% of the power scattered by gray matter was scattered within angles smaller than about 3 degrees. The error was between 10 and 25% from smaller to larger values of the scattering angle.

The smallest value for τ_m obtained for measurements of scattering functions of white matter was 3. A strong error due to multiple scattering is thus expected. Measurements of scattering function carried out on samples of calibrated polystyrene spheres at different optical thicknesses enabled us



Fig. 7 Scattering functions for white matter measured on (a) three samples of swine and (b) on four samples of bovine brain. The optical thickness for the swine samples was 3 for sample 1, 3.8 for sample 2, and 5.6 for sample 3. The optical thickness for the bovine samples was 3, 4.2, 5, and 3.8 for samples 1, 2, 3, and 4 respectively.

to make a rough estimate of the error due to multiple scattering effects. As an example, from measurements carried out on spheres with Φ =11.9 μ m at τ_m =3.7, we obtained a scattering function very similar to the one measured on sample 2 of white matter of swine at τ_m =3.8. The scattering function



Fig. 8 The measured scattering functions for white matter are overestimated due to multiple scattering effects. The figure shows (filled circles) the measured scattering function for white matter of swine brain (sample 2). The figure also shows the values (hollow circles) obtained by applying the correction factors to take into account multiple scattering effects evaluated from measurements on polystyrene spheres.

of spheres was overestimated by a factor of 1.7 at ϑ =0.2 degrees and by a factor of 12 at ϑ =2.6 degrees. In Fig. 8 the values of the scattering function for the sample of brain obtained by dividing the measured values by the correction factor obtained from measurements on polystyrene spheres are shown.

For all samples, the results obtained showed highly forward-peaked scattering functions, indicating large values for the asymmetry factor. Calculations with Mie theory showed that values for the scattering function in the forward direction similar to the one obtained for gray matter are obtained for spheres with a diameter of $\approx 35 \ \mu m$ when a relative refractive index of 1.04 with respect to the surrounding medium is assumed for the diffusers, as can be expected for brain tissue.⁴ For these spheres, a large asymmetry factor ($g \cong 0.99$) is obtained. This estimation of g was obtained with very rough assumptions: the scattering particulate was assumed to be spherical and the comparison of the scattering function was possible only for $\vartheta < 2.6$ degrees. The value of *g* indicated is thus a rough approximation.

8 DISCUSSION

8.1 EXTINCTION COEFFICIENT

With regard to extinction coefficient, some data referring to brain tissue at λ =633 nm obtained using different methods are reported in the literature. Wilson, Patterson, and Burns⁷ reported for porcine brain $\mu_e = 103.7 \text{ mm}^{-1}$, which was obtained by using an indirect method based on diffusion theory, but the kind of matter (gray or white) was not specified. The same information was also lacking in a paper by Flock, Wilson, and Patterson⁸ where a value of μ_e =68.7 mm⁻¹ for porcine brain obtained by using the Lambert-Beer law was reported. Splinter et al. reported for human brain a $\mu_e = 5.3 \text{ mm}^{-1}$ for white matter and 6.3 mm⁻¹ for gray matter, which was derived by applying the Kubelka-Munk 3-flux model to transport theory. Splinter et al. also reported values of 9.2 mm⁻¹ and 5.8 mm⁻¹ respectively for white and gray matter of canine samples. For human adult brain, Van der Zee, Essenpreis, and Delpy⁴ reported μ_e =62 mm⁻¹ for gray matter and $\mu_e=47 \text{ mm}^{-1}$ for white matter. These values were obtained from measurements of the diffuse reflectance and transmittance of light, by thick samples. Values significantly smaller than ours have been recently reported by Jarry et al.;¹⁰ by using heterodyne detection they measured the extinction coefficient of sheep brain at 790 nm. For gray matter they obtained values of μ_e =3.42 mm⁻¹ and for white matter $\mu_e = 3.14 \text{ mm}^{-1}$. Measurements were carried out on samples 2 to 7 mm thick.

Differences among the results reported in different papers can be due to different measurement methods (direct and indirect) used.

8.2 PHASE FUNCTION

To our knowledge, no data on the single scattering function in the small scattering angle range have been established. Few research groups have carried out measurements of $p(\vartheta)$ in the range ≈ 2 to 170 degrees so not many sets of data on $p(\vartheta)$ can be found in the literature. A knowledge of $p(\vartheta)$ at small scattering angles is important for media having a pronounced forward scattering, i.e., an asymmetry factor very close to 1, since about 50% of the power is scattered within ϑ smaller than about 3 degrees.

Experimental results on human brain tissues have been reported by Van der Zee, Essenpreis, and Delpy.⁴ Goniometric measurements of the phase function are reported both for white and for gray matter. These data have been used to obtain the asymmetry factor. Like our results, the values of $p(\vartheta)$ near 0 degrees measured by these authors showed a highly forward scattering; also the scattering function for white matter is forward peaked, but no information is given on the optical thickness of the slice used. Our measurements showed the impossibility of measuring the single scattering phase function for white matter, since the optical thickness obtained was significantly larger than 1.

9 CONCLUSIONS

The results of measurements carried out to obtain optical properties of biological tissues have been presented. Measurements of single scattering properties (extinction coefficient and scattering function at small scattering angles) were carried out on thin samples of swine and bovine brain. Experimental results showed that the extinction coefficient for white matter is about 9 times larger than for gray matter.

Measurements of scattering function showed a strong forward peak especially for gray matter: more than 50% of scattered power was emitted within 3 degrees. The extinction coefficient for white matter was so high (it was impossible to obtain samples with optical thicknesses smaller than about 3) that the results obtained for the phase function were strongly affected by multiple scattering error. The white matter of adult swine and cows is thus such a strongly packed diffusing medium that it is impossible to obtain a direct measurement of the single scattering function. Small differences were found between the optical properties of swine and bovine samples.

Measurements were carried out at room temperature (20 to 24°) on thin slices of brain 2 to 5 hours postmortem. Thus the results obtained may not necessarily provide an accurate representation of *in vivo* optical properties, since the scattering properties may vary postmortem or during the sample preparation. However, the results presented can be useful in developing models for light propagation through the brain. The models are useful, for instance, for imaging.

Acknowledgments

This research was supported in part by Consiglio Nazionale delle Ricerche (CNR) grant No. 93.03342.CT 11.

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