Optical absorption and scattering properties of bulk porcine muscle phantoms from interstitial radiance measurements in 650–900 nm range

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Abstract
We demonstrated the application of relative radiance-based continuous wave (cw) measurements for recovering absorption and scattering properties (the effective attenuation coefficient, the diffusion coefficient, the absorption coefficient and the reduced scattering coefficient) of bulk porcine muscle phantoms in the 650–900 nm spectral range. Both the side-firing fiber (the detector) and the fiber with a spherical diffuser at the end (the source) were inserted interstitially at predetermined locations in the phantom. The porcine phantoms were prostate-shaped with ∼4 cm in diameter and ∼3 cm thickness and made from porcine loin or tenderloin muscles. The described method was previously validated using the diffusion approximation on simulated and experimental radiance data obtained for homogenous Intralipid-1% liquid phantom. The approach required performing measurements in two locations in the tissue with different distances to the source. Measurements were performed on 21 porcine phantoms. Spectral dependences of the effective attenuation coefficient and absorption coefficients for the loin phantom deviated from corresponding dependences for the tenderloin phantom for wavelengths <750 nm. The diffusion constant and the reduced scattering coefficient were very close for both phantom types. To quantify chromophore presence, the plot for the absorption coefficient was matched with a synthetic absorption spectrum constructed from deoxyhemoglobin, oxyhemoglobin and water. The closest match for the porcine loin spectrum was obtained with the following concentrations:
15.5 μM (±30% s.d.) Hb, 21 μM (±30% s.d.) HbO₂ and 0.3 (±30% s.d.) fractional volume of water. The tenderloin absorption spectrum was best described by 30 μM Hb (±30% s.d), 19 μM (±30% s.d.) HbO₂ and 0.3 (±30% s.d.) fractional volume of water. The higher concentration of Hb in tenderloin was consistent with a dark-red appearance of the tenderloin phantom. The method can be applied to a number of biological tissues and organs for interstitial optical interrogation.

Keywords: porcine muscle, radiance spectroscopy, optical properties, absorption, scattering, tissue oxygenation

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(Some figures may appear in colour only in the online journal)

1. Introduction

Biological phantoms made from various animal tissues play an important role in biomedical optics research (Cheong et al 1990, Bashkatov et al 2011). As oppose to homogenous liquid phantoms like Intralipid-based or similar, they demonstrate a degree of heterogeneity and morphological complexity common for biological tissues. Also, they have a set of major chromophores that is shared by human tissues. In addition, they are relatively easy available to most research labs which do not have an access to human tissues and organs through collaborations with medical centers or hospitals.

Knowledge of optical properties of biological phantoms or tissues is extremely important because ‘...specifying the optical properties of a tissue is the first step toward properly designing devices, interpreting diagnostic measurements or planning therapeutic protocols’ (Jacques 2013). Swine are considered to be one of the major animal species used in translational and basic research because they share with humans similar anatomic and physiologic characteristics involving the cardiovascular, urinary, integumentary, and digestive systems (Swindle et al 2012). It is not surprising that they are increasingly being used as an alternative to dog or monkey. They are excellent models for toxicological testing (Swindle et al 2012), cardiovascular disease (Turk et al 2005), hypertension (Dyson et al 2006), diabetes (Larsen and Rolin 2004), obesity (Dyson et al 2006), intestinal function (Domenechini et al 2006), cystic fibrosis (Rogers et al 2008), nutrition (Mitchell 2007), skin physiology (Simon and Maibach 2000), injury and repair (Winter 2006), breast cancer ablation (Robinson et al 1998), prostate diagnostics (Grabtchak et al 2013). Porcine muscle tissues can serve as versatile biological phantoms but unfortunately, not much data on their optical properties are available in the literature. Most known data were obtained at a single wavelength from in vitro double-integrating sphere (Cheong et al 1990, Beek et al 1997) or diffuse reflectance measurements (Sun and Wang 2010, Alali et al 2012).

However, spectroscopic capabilities have been proven to be extremely powerful in biomedical research (Rolfe 2000, Hoshi 2011). Spectrally resolved optical properties not only provide data for wavelengths of possible interest, but allow relating spectral signatures to the presence of certain tissue chromophores (Doornbos et al 1999, Bargo et al 2005). Moreover, concentration of hemoglobin and tissue oxygenation are important biomarkers that can serve as indicators in diagnostics of a wide variety of diseases (Ballin et al 1995, Cortez et al 2011, El-Desoky et al 2001, Gareau et al 2010, Gay et al 2011, Hansen et al 2013,
Jhanji et al 2009, Shapiro et al 2011) including cancer (Brizel et al 1996, Krause et al 2006, Vaupel et al 2003). In spite of accumulated knowledge (Cheong et al 1990, Bashkatov et al 2011), spectroscopic studies of optical properties of animal tissues still do not share the same degree of details and comprehensiveness as human tissues (Sandell and Zhu 2011). Hence, it appears to be some gap between tremendous potentials of animal phantoms as model systems for human tissues and the level of encompassing knowledge of their actual properties. For example, the lack of needed data of optical properties of porcine muscles may force researchers to use optical properties of other biological tissues as in the work on modeling of coagulation effects (Mohammed and Verhey 2005). The current work attempts to address this issue for porcine muscle tissues.

In the earlier studies we introduced an experimental method for extracting absorption and scattering properties (the effective attenuation coefficient, the diffusion coefficient, the absorption coefficient and the reduced scattering coefficient) of turbid media based on the diffusion approximation from relative spectrally resolved continuous wave (cw) radiance measurements (Grabtchak and Whelan 2012). The method was validated on the Intralipi-1% liquid phantom. In the current work we demonstrate the first interstitial application of the method to biological phantoms (21 in total) made from porcine muscle tissues (pork loin and pork tenderloin). Spectrally resolved data allowed obtaining optical properties of tissues in the wide spectral range (∼650–900 nm) and relate the measured absorption to contributions of deoxyhemoglobin, oxyhemoglobin and water. The effect of the fiber positioning tolerance on the measured values was analyzed. Current study also complements the earlier work on detection of localized inclusions of gold nanoparticles in the prostate-shaped porcine muscle phantoms (Grabtchak et al 2013).

2. Materials and methods

2.1. Experimental set-up for radiance measurements

The experimental set-up used in the current work has been described in great details in the number of publications (Grabtchak et al 2011, 2012). Therefore, only a brief description is given here. A schematic of the experimental set-up is shown in figure 1.

Illumination was achieved with 20 W tungsten halogen white light source (Ocean Optics) that was connected to a fiber with 2 mm spherical diffuser at the end. A 600 µm side firing fiber (Pioneer Optics) served as a detector and was connected to a miniature spectrometer USB 4000 (Ocean Optics). The detecting fiber was held in a computer-controlled rotation stage that provided the full 360° rotation with 0° angle corresponding to detector facing the source. Illumination and detection was performed interstitially by inserting the fibers into the tissue at predetermined locations. Both illuminating and detecting fibers were threaded through 15 gauge needles for mechanical stability but only the protruding part of the detecting fiber was inserted to the phantom to reduce self-scattering effects discussed in Grabtchak et al (2012). All interstitial measurements required puncturing the phantom with 15 and 17 gauge needles to produce holes that would accommodate the illuminating and detecting fiber, correspondingly. In all phantoms the illuminating fiber was always placed ∼5 mm away from the edge of the phantom while the detecting fiber was inserted at various distances from the illuminating fiber. The porcine phantom was held in a black plastic holder with internal diameter of ∼40 mm. The inset on figure 1 shows a representative top-view image of the porcine phantom with the marked illumination point and two measurement points delineated with the circles and distances to the illumination point. The distances were measured with a ruler. Radiance data were acquired by rotating the side firing fiber over a 360° range with a
Figure 1. The schematic of the experimental set-up for interstitial radiance measurements. Inset: the top view of the representative porcine phantom in the holder with locations of the measurements and illumination points.

2\textsuperscript{o} step and collecting spectra with USB 4000 at every angular step. It took about 4 min to acquire a complete single angular profile containing 180 spectra.

2.2. Optical properties extraction algorithm

The new method for extracting the effective attenuation coefficient and the diffusion coefficient of turbid media from relative spectrally resolved cw radiance measurements using the diffusion approximation was validated on both simulated and experimental radiance data sets using Intralipid-1% liquid phantom as a test platform (Grabtchak and Whelan 2012). Hence, only final formulas will be given here. The effective attenuation coefficient, $\mu_{\text{eff}}(\lambda)$ is determined from a simple algebraic expression constructed from a ratio of two radiance measurements at two different source–detector separations ($r_0$ and $r$) and the same 90\textdegree angle:

$$
\mu_{\text{eff}}(\lambda) = \ln \left( \frac{I(r, 90^\circ, \lambda) \cdot r}{I(r_0, 90^\circ, \lambda) \cdot r_0} \right) / (r_0 - r)
$$

Then, with the knowledge of $\mu_{\text{eff}}(\lambda)$ the diffusion coefficient, $D(\lambda)$ is determined from another ratio constructed from two radiance measurements at two angles (0\textdegree and 180\textdegree) and the same source–detector separation:

$$
D(\lambda) = (1 - I(r, 180^\circ, \lambda)/I(r, 0^\circ, \lambda)) / (3(\mu_{\text{eff}}(\lambda) + 1/r)(1 + I(r, 180^\circ, \lambda)/I(r, 0^\circ, \lambda)))
$$

Once $\mu_{\text{eff}}(\lambda)$ and $D(\lambda)$ are obtained, the absorption coefficient, $\mu_a(\lambda)$ and the reduced scattering coefficient, $\mu'_s(\lambda)$ can be determined from them as follows:

$$
\mu_a(\lambda) = \mu_{\text{eff}}^2(\lambda) \cdot D(\lambda),
$$

$$
\mu'_s(\lambda) = (1 - 3 \cdot \mu_{\text{eff}}^2(\lambda) \cdot D^2(\lambda)) / 3 \cdot D(\lambda)
$$

By reducing a diameter of the section of the fiber inserted in the tissue (bare fiber versus fiber in a needle), the systematic error of the method was reduced down to $\sim10\%$. Plots for all four parameters are presented in the results section.
2.3. Porcine samples

Porcine meat (pork loin centre roast, boneless) was purchased at a local grocery store in ∼0.8–1.0 kg packages on a day they were packaged (see figure S1, available from stacks.iop.org/PMB/59/2431/mmedia). In total, five different packages were used in experiments. Following the phantom geometry used in Grabtchak et al (2013), the porcine phantom was prepared by cutting a prostate-size piece (∼4 cm diameter, ∼3 cm thick) from a larger piece of meat. In average, every package produced 4–5 phantoms. Overall, measurements were performed on 21 porcine phantoms. When not in use, the remaining porcine meat was stored in a fridge at +4 °C. Each large package was consumed in ∼3 days. On a single occasion, a different part of pork, tenderloin was used for measurements. According to porcine anatomy, the loin part corresponds to Longissimus dorsi (LD) muscles, while the tenderloin part is composed of Psoas major (PM). All individual phantoms were always cut perpendicular to muscle fibers.

Tenderloin is easily distinguished from loin by a darker meat appearance. Some representative images of the phantoms are shown in figure 2. One can see that all shown samples have different degrees of heterogeneity formed by a network of white fatty inclusions.
Figure 3. Effective attenuation coefficient for 21 loin and single tenderloin phantoms.

Hence, none of the measured phantoms can be considered identical but represent a typical morphological diversity of the porcine muscle (loin) tissue.

3. Results and discussion

Various source–detector separation pairs were used in the experiments with the corresponding number of phantoms shown in brackets: 10 and 20 mm (11), 18 and 24 mm (2), 10 and 18 mm (2), 15 and 25 mm (2), 10 and 24 mm (2), 7 and 15 mm (2). We did not observe any correlation between calculated optical parameters and different source–detector separations. Also, we did not see any correlation between data obtained for phantoms that were cut from the same large porcine piece. Therefore, all 21 measurements represent uncorrelated and random sampling of porcine muscle tissue.

The distribution of measurements \(n = 21\) of \(\mu_{\text{eff}}(\lambda)\) for the porcine phantom (loin) is presented in figure 3 along with a single measurement taken from the porcine tenderloin phantom (shown with a cyan dash–dot line). The shape of all 21 curves is very close to each other in spite of the vertical offset. Figures 4(a)–(d) show the calculated mean and the standard deviation (gray area) for \(\mu_{\text{eff}}(\lambda), D(\lambda), \mu_a(\lambda)\) and \(\mu'_s(\lambda)\) for porcine loin phantoms as well as the single measurement for the porcine tenderloin phantom. One can notice that \(\mu_{\text{eff}}(\lambda)\) and \(\mu_a(\lambda)\) for the loin show the spectral behavior that differs from that one for the tenderloin phantom for wavelengths <750 nm. However, \(D(\lambda)\) and \(\mu'_s(\lambda)\) are very close for both phantom types. It indicates that chromophore differences in loin and tenderloin phantoms are manifested mainly in the absorption properties while scattering is unaffected by it and remains approximately the same.

Because of a large source–detector separation, photons interact with a large portion of the phantom before reaching the detector. Thus, various heterogeneities present in the volume contribute to averaged optical properties of the phantom. Also, since all phantoms were cut across muscle fibers various in-plane orientations of the source and detector did not reveal any in-plane anisotropy in optical properties. It is possible that for phantoms cut along muscle
fibers, two orthogonal positions of the source–detector pair (along the fibers versus across the fibers) would reveal some anisotropy in the mean values of optical properties.

In order to quantify differences in chromophore concentrations in porcine loin and tenderloin phantoms, we attempted to fit the measured $\mu_a(\lambda)$ with a synthetic absorption spectrum consisting of three chromophores: deoxyhemoglobin (Hb), oxyhemoglobin (HbO2) and water as

$$\mu_a/\Sigma_1 = \ln(10) \cdot (\epsilon_{\text{Hb}} \cdot C_{\text{Hb}} + \epsilon_{\text{HbO2}} \cdot C_{\text{HbO2}}) + f \cdot \mu_{a\text{H2O}},$$

where $\epsilon$ is the molar extinction coefficient, $C$ is the concentration, $f$ is the volume fraction of water and $\mu_{a\text{H2O}}$ is the absorption coefficient of water. The nonlinear least-square fitting algorithm was implemented in OriginPro 8.1 (OriginLab Corp.). The expression for the absorption coefficient of the tissue, $\mu_a/\Sigma_1$ is a linear combination of multiple chromophores and can be expressed in a number of equivalent ways (Jacques 2013). Since absorption properties of water are usually presented in terms of the absorption coefficient (base e nomenclature) rather than the molar extinction coefficient (base 10 nomenclature), the expression for $\mu_a/\Sigma_1$ was adapted for this purpose.

The absorption spectrum of water was taken from Segelstein (1981). For the molar extinction coefficients of Hb and HbO2 we used slightly different spectra from Prahl (1998), Takatani and Graham (1987). As one can conclude from the collection of spectra presented at the web page of Prahl, in spite of a general agreement some minute differences can make certain spectra look better than the others in the composite spectrum. Such synthetic absorption spectra along
Figure 5. Absorption coefficient and the synthetic absorption spectrum for (a) loin phantom and (b) tenderloin phantom.

with the absorption spectra of loin and tenderloin phantoms are shown in figures 5(a) and (b) correspondingly.

The closest match for the porcine loin spectrum was obtained using the spectra from Takatani and Graham (1987) with the following concentrations: 15.5 μM (± 30% s.d.) Hb, 21 μM (± 30% s.d.) HbO2 and 0.3 (± 30% s.d.) fractional volume of water (shown with dashed red line in figure 5(a)). Recreated synthetic absorption spectrum using the same parameters with the spectra from Prahl (1998) is shown with dash–dotted blue line in figure 5(a). One can notice that Prahl’s spectra show a poorer match especially in the spectral range <700 nm. Adding the fourth chromophore, fat to the synthetic spectrum did not improve the agreement with the experimental curve. The synthetic absorption spectrum for the tenderloin phantom that produced the best match to the experimental data was obtained with spectra from Takatani and Graham (1987) and the following concentrations: 30 μM Hb (± 30% s.d), 19 μM (± 30% s.d.) HbO2 and 0.3 (± 30% s.d.) fractional volume of water (shown with dashed red line in figure 5(b)). The higher concentration of Hb in tenderloin is consistent with a dark-red appearance of the tenderloin phantom as oppose to a lighter shade of the loin phantom. The total concentration of hemoglobin is also higher in the tenderloin phantom (49 versus 36.5 μM in the loin), while tissue oxygenation is higher in the loin phantom (57% versus 39% in the tenderloin).

Understanding and proper interpretation of these results require invoking some general concepts pertinent to post mortem tissues (or meat). Myoglobin (Mb) and hemoglobin (Hb) are the principle hemoproteins giving muscles and meat the red color (Govindarajan and Snyder 1973, Mancini and Hunt 2005). The estimated contribution of myoglobin to hemoglobin levels derived from near-infrared spectroscopy (NIRS) is unclear. Reports have ranged from suggesting that nearly all of NIRS-derived signal is from Mb (Tran et al 1999) to suggesting that 90% of the signal is coming from Hb (Mancini et al 1994, Boushel and Piantadosi 2000). In biological tissues, NIR light is specifically absorbed by heme groups within Hb and Mb. The absorption spectra of Hb and Mb are, for practical purposes, indistinguishable in the near infrared region (Schenkman et al 1999, van Beekvelt et al 2001). Thus NIRS cannot differentiate between the two species. Regardless of Mb contribution to NIRS signals, it is customary to consider NIRS measurements as indexes of overall tissue oxygenation (van Beekvelt et al 2001, Subudhi et al 2007, Shaw et al 2000, Gussakovsky et al 2008, Myers et al 2009).
The changes that occur in excised muscles with a permanently terminated blood perfusion not only take place on a different time scale than those during short ischemic cycles in live tissues (Matcher et al 1995, Myers et al 2009) but also follow different metabolic and biochemistry patterns. The different myoglobin species in meat include deoxymyoglobin (Mb, purple color), oxymyoglobin (MbO2, bright cherry red) and metmyoglobin (MetMb, brown) (Govindarajan and Snyder 1973, Mancini and Hunt 2005). Myoglobin has high affinity for oxygen. Very low oxygen partial pressure, <1.4 mm Hg (Brooks 1935) is required to maintain Mb in a deoxygenated state. Mb is nearly fully saturated at an oxygen partial pressure of 10 mm Hg (George and Stratmann 1952). Thus, under air-saturated conditions almost all Mb exists in the oxy-form, MbO2. The ferrous species Mb and MbO2 oxidize to ferric MetMb upon which the oxygenation ability is lost and meat acquires brown color. For freshly cut meat, the myoglobin is in the reduced form Mb. On exposure to air, Mb within minutes reversibly combines with oxygen to form the bright cherry-red MbO2 following the reaction known as blooming (Ledward 1992). As exposure to oxygen increases, MbO2 penetrates deeper beneath the meat’s surface. Depth of oxygen penetration and thickness of the MbO2 layer depend on the meat’s temperature, oxygen partial pressure, pH, and the activity of the oxygen-consuming enzymes (Ledward 1992, Mancini and Hunt 2005). Oxygen uptake in post mortem muscles is a result of tissue respiration, reaction with hemoproteins and dissolution into tissue fluids (Devore and Solberg 1974). Even in post mortem muscles mitochondria continue to metabolize oxygen (Ashmore et al 1971, 1972, Tang et al 2005, Devore and Solberg 1974, Lanari and Cassens 1991). However, the oxygen consumption rate declines with time post mortem in tissues due to loss of structural integrity of the mitochondria (Cheah and Cheah 1971). It allows oxygen to penetrate further into the muscle (Millar et al 1994).

Hence, high values of post mortem tissue oxygenation are expected. The percentage of deoxymyoglobin, oxymyoglobin, and metmyoglobin is usually determined at the meat surface by measuring optical reflectance at selected wavelengths according to the method of Krzywicki (Krzywicki 1979) or its variations. The relative percentage of MbO2 was found to be in 69–80% range for LD (measured three days post mortem) for different pig breeds indicating a high level of tissue oxygenation (Lindahl et al 2001). High relative percentage of MbO2, 72.59% and 64.07% were reported in Longissimus thoracis, and Masseter porcine muscles determined in four days post mortem and after two days of air exposure (Realini et al 2013). It was also shown that relative percentage of MbO2 decreased from 72% (first day) to ∼68% for porcine LD and ∼57% for porcine PM in five days post mortem (Jeong et al 2009).

Thus, values of tissue oxygenation (57% for LD and 39% for PM) presented in the current work are consistent with high values reported in the literature but are still lower. The observed differences might be due to a number of reasons: (1) as oppose to surface measurements of Krzywicki method, our measurements were performed in the bulk tissue at ∼17 mm below the surface where oxygenation level may be lower; (2) due to the diffuse nature of photon propagation, the measured signal includes contributions from the entire volume of the tissue; (3) the usage of the full spectral range (650–900 nm) for chromophores identification may provide more accurate results than using only few discrete wavelengths.

The higher concentration of the total hemoglobin in PM (49 μM) versus LD (36.5 μM) is also expected. Muscles can be classified as glycolytic (white) or oxidative (red) based on the proportion of glycolytic and oxidative fiber types in the muscle (Beecher et al 1965). White muscles contain glycogen and enzymes related to glycolysis while oxidative muscles have a large amount of myoglobin for oxygen storage and high numbers of mitochondria. It is known that PM is oxidative, and LD is glycolytic (Chang et al 2003). As compared with white muscles, higher hemoprotein content in red muscles was reported in a number of publications.
Water fractional volume appears to be about 0.3 (or \(\sim 30\%\)) in both phantoms which is lower than the expected 0.6–0.7 range for \textit{in vivo} tissues. While water content in excised tissues will depend on tissue handling and time elapsed after extraction, it is plausible that the measured water content might be somewhat underestimated. The reason for a possible underestimation can be a limited spectral range where water concentration makes a noticeable contribution. Extending the spectral range up to \(\sim 1000 \text{ nm}\) would include the first water absorption peak and make the measurement more accurate. This can be accomplished by using a more powerful light source.

Unfortunately, not many data on optical properties of porcine muscle tissues are available in the literature. Most known measurements were done at a fixed wavelength: \(\mu_a = 0.1 \text{ mm}^{-1}\), \(\mu_s' = 0.12 \text{ mm}^{-1}\) (633 nm, integrating sphere) (Cheong \textit{et al} 1990); \(\mu_a = 0.059 \pm 0.001 \text{ mm}^{-1}\), \(\mu_s' = 2.47 \pm 0.07 \text{ mm}^{-1}\), \(\mu_{\text{eff}} = 0.67 \pm 0.01 \text{ mm}^{-1}\) (632.8 nm) and \(\mu_a = 0.12 \pm 0.001 \text{ mm}^{-1}\), \(\mu_s' = 6.21 \pm 0.2 \text{ mm}^{-1}\), \(\mu_{\text{eff}} = 1.5 \pm 0.07 \text{ mm}^{-1}\) (630 nm) (integrating sphere) (Beek \textit{et al} 1997); \(\mu_{\text{eff}} = 0.1–0.22 \text{ mm}^{-1}\), \(\mu_s' = 0.16–0.18 \text{ mm}^{-1}\), \(\mu_a = 0.055–0.065 \text{ mm}^{-1}\) (650 nm, diffuse reflectance) (Sun and Wang 2010); \(\mu_a = 0.037 \pm 0.004 \text{ mm}^{-1}\), \(\mu_s' = 0.4 \pm 0.007 \text{ mm}^{-1}\) (635 nm, diffuse reflectance) (Alali \textit{et al} 2012). Out of these data, values from diffuse reflectance measurements appear to be closer to ours. In particular, \(\mu_{\text{eff}} = 0.1–0.22 \text{ mm}^{-1}\) (Sun and Wang 2010) and \(\mu_s' = 0.4 \pm 0.007 \text{ mm}^{-1}\) (Alali \textit{et al} 2012) match our corresponding data. While the observed differences can be due to different parameter extraction algorithms and calibration aspects, another important aspect is freshness of the sample. It was suggested that post-mortem tissue storage produces a reduction in \(\mu_a\) and an increase in \(\mu_s'\) due to losses of hemoglobin (Roggan \textit{et al} 1999). Thus, a comparison of various results obtained without clear knowledge of sample’s handling history and a correlation between storage time and hemoglobin concentration may not be justified. For the samples used in the current work, duration of sample’s handling in the lab (1–3 days) dominated prior several hour storage (estimated) in a grocery store.

The accuracy of determination of optical properties depends also on the accuracy of the source–detector distance measurements. The typical accuracy of distance measurements was \(\pm 0.5 \text{ mm}\). However, since the method requires calculating the difference between the two source–detector separations, we investigated how the offset would affect the final values. For this purpose, calculations were performed initially for a selected data set obtained at nominal 15 and 25 mm source detector separations. Then, considering the errors adding up in distance measurements we repeated calculations using 14.5 and 25.5 mm distances (+ 1 mm offset) and 15.5 and 24.5 mm (−1 mm offset). The calculated optical parameters marked as ‘nominal’, ‘+1 mm offset’ and ‘−1 mm offset’ are shown in figures 6(a)–(d) along with the mean and standard deviation values.

One can see that the offset in distance measurements produces values that are in the range of data obtained from multiple phantom measurements. Hence, the presented data may reflect not only the variation of optical properties from phantom-to-phantom but possible accumulation of the error in distance measurements.

The demonstrated method can be applied to a variety of biological phantoms or organs for determining the absorption and scattering properties. Since the measurements are performed interstitially at only two locations inside the bulk tissues, it minimizes a degree of invasiveness while taking into account possible heterogeneity of bulk tissues. Large source–detector separations ensure that photons sample a significant region of the bulk tissue. The method does not require complex equipment or calibrations for absolute measurements which may open avenues for its clinical applications.
4. Conclusions

In conclusion, we have obtained the optical properties (the effective attenuation coefficient, the diffusion coefficient, the absorption coefficient and the reduced scattering coefficient) of bulk porcine muscle phantoms (loin and tenderloin) in the 650–900 nm spectral range using relative cw interstitial radiance measurements. Spectrally resolved absorption coefficient was quantified in terms of contributions from absorption of deoxyhemoglobin, oxyhemoglobin and water. It allowed calculating the total hemoglobin concentration and the oxygenation level of the tissue. Because the absorption spectra of hemoglobin and myoglobin are practically indistinguishable in 650–900 nm spectral range, the obtained values should be interpreted as concentrations of hemoproteins still giving the correct levels of tissue oxygenation. Absorption coefficient of the loin phantom was found to be lower than that one of the tenderloin phantom while the reduced scattering coefficient was very similar for both types of phantoms. The effect of a possible uncertainty in distance measurements was investigated. The presented method can be applied to a number of biological tissues and organs for interstitial optical interrogation.
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