Monitoring sarcomere structure changes in whole muscle using diffuse light reflectance

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Abstract. Normal biomechanical and physiological functions of striated muscles are facilitated by the repeating sarcomere units. Light scattering technique has been used in studying single extracted muscle fibers. However, few studies, if any, have been conducted to investigate the possibility of using optical detection to examine sarcomere structure changes in whole muscles. We conducted a series of experiments to demonstrate that optical scattering properties measured in whole muscle are related to changes in sarcomere structure. These results suggest that photon migration technique has a potential for characterizing *in vivo* tissue ultrastructure changes in whole muscle. © *2006 Society of Photo-Optical Instrumentation Engineers.* [DOI: 10.1117/1.2234278]

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Skeletal muscle contributes to 40% of total human weight and is responsible for many important physiological functions. In addition, skeletal muscle's striated relative, cardiac muscle, is essential to life by controlling circulation under various physiological conditions. Sarcomeres are the fundamental structural and contractile units in striated muscle and sarcomere length is an important predictor of muscle function.[1] Many muscle diseases ultimately are reflected in changes in sarcomere organization.[2]

Sarcomeres are aligned precisely in muscle fibers and are readily observed using light microscopy as alternating light and dark bands called the I-band and A-band. The striated appearance of myofibrils makes laser diffraction[3] an ideal method to study sarcomere dynamics in single muscle fibers. However, because of the multiple scattering that occurs as well as the added complexity provided by other tissue-specific components, a light diffraction pattern is not discernable in thick muscle samples. Therefore, it is not clear whether sarcomere changes can still be monitored in whole muscle.

Tissue scattering is related to the morphology and refractive index distributions of the tissue composition. Because the periodic sarcomere structures can significantly alter light propagation directions in muscle, we hypothesize that changes in sarcomere structure can be monitored in whole muscle by measuring the scattering coefficient. Although whole muscle scattering coefficients have been measured before,[4] the effects of sarcomere structure changes have not been studied. In this paper, we study the reduced scattering coefficients derived from spatially resolved reflectance measurements[5] on pre- and postrigor whole muscles and investigate their relationships with sarcomere structure.

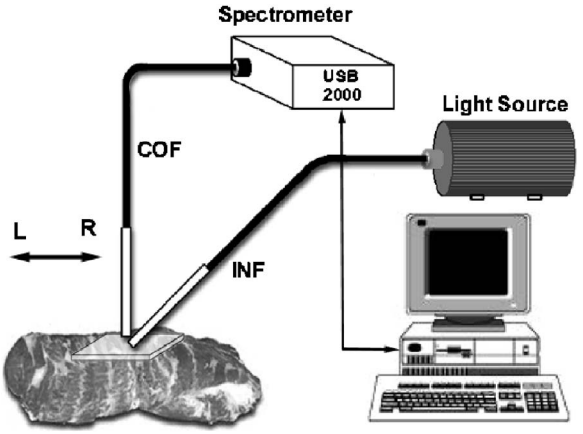

Fig. 1 Instrumentation for the spatially resolved reflectance measurement. COF: collection fiber; INF: incident fiber.

Beef *sternomandibularis* and *psoas major* muscles were used in this study. Whole *sternomandibularis* muscles (~5 × 20 × 5 cm in width × length × thickness, respectively) were excised from beef carcasses immediately (within 1 min postexsanguination) after slaughter and external fat was removed. We measured scattering coefficients of these samples immediately. To manipulate muscle sarcomere length, the *psoas major* muscles were used because of their appropriate anatomical locations. Specifically, muscles were either left on the carcasses to induce longer sarcomere lengths,[6] or were removed and subjected to reduced ambient temperatures, which are known to result in shorter sarcomere lengths.[7] The removed muscles were wrapped in plastic wrappers. After completion of rigor mortis (~24 h), we measured scattering coefficients of these samples. All optical measurements were conducted in the sample storage room (temperature 4 °C and 70% humidity).

Muscle optical scattering properties were measured using the oblique-incidence reflectometry method.[5] This method can measure sample absorption coefficients μ_a and reduced scattering coefficients $\mu_s' = \mu_s(1-g)$, where μ_s is the scattering coefficient and g is the anisotropy.

The experimental apparatus is shown in Fig. 1. A 20-W broadband halogen light (HL-2000-FHSA-HP, Ocean Optics Inc., Dunedin, Florida) was used as the light source. Light was incident upon the sample through a 400-μm-diameter optical fiber (INF) at an oblique incident angle. The incident angle used was 28 ~29 deg at muscle surface. A second 400-μm fiber (COF) was mounted at 90 deg to the sample surface to collect the diffuse reflectance. It was mounted on a translation stage and scanned above the sample to measure the spatially resolved reflectance. The scanning plane of the collection fiber was parallel to the plane of incidence, but was

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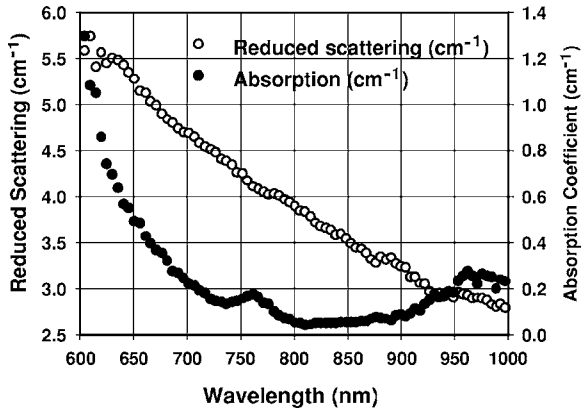

Fig. 2 Example of measured muscle absorption and reduced scattering spectra.

offset by a small distance of 1.5 mm to avoid collision with the incident fiber. Sample surface was mounted against a black plate with a slit window made of a cover glass for scanning. All fibers were positioned slightly above the slit window. The collection fiber was connected to a spectrometer (USB2000, Ocean Optics Inc.). Reflectance spectra were collected from 12 positions, and ranged from 9.0 to 6.5 mm on the left (L in Fig. 1) and from 4.0 to 6.5 mm on the right (R in Fig. 1) relative to the incident location at an interval of 0.5 mm. Spectral data from 600 to 1000 nm wavelengths were processed to derive μ_a and μ_s' by using a diffuse fitting method that has been described in detail elsewhere.[5] Although only the 721-nm wavelength data are shown below (except for Fig. 3(a)), other wavelengths from 600 to 900 nm produced similar results. Results at wavelengths greater than 900 nm had large fluctuations caused by small signal intensities due to low grating efficiency.

The experimental system was validated using standard scattering phantoms made from 20% Intralipid (Sigma-Aldrich, Inc., St. Louis, Missouri) and the measurement errors were found to be within 10% of the true values. Previous study has shown that the reduced scattering coefficients measured on chicken breast tissues were affected by the muscle fiber orientations.[5] In the current study, all measurements were conducted with the fiber scanned along the axis perpendicular to the long axis of the muscle fibers.

Figure 2 shows an example of measured absorption and reduced scattering spectra of a muscle sample (*psoas major*). The reduced scattering coefficient decreases with wavelength. The absorption coefficient decreases significantly from 600 to 750 nm. The small absorption peak at ~760 nm is coincident with the absorption peak of deoxymyoglobin and deoxyhemoglobin. Because of the exsanguination process, myoglobin has been considered as the dominant pigment in beef[6] although any residual blood may also contribute to the absorption. Within the bandwidth of 650 to 1000 nm, the muscle scattering is dominant so that diffuse approximation can be applied.

Figure 3(a) shows the changes in scattering coefficients of muscle samples during rigor mortis development. Whole *sternomandibularis* muscles were mounted on our experiment setup by restraining the two ends, thereby fixing muscle length during the entire duration of the experiment. Reduced scattering coefficients were measured continuously at the same location on the muscle every 5 min. Results in Fig. 3(a) are reported as average values over three different animals (muscles). Initial (zero time) values possessed considerable variations. The initial values at 721 nm are 3.06, 2.44, and 3.09 cm^{-1}; and they were 2.54, 2.09, and 2.58 cm^{-1} at 853 nm. To avoid such muscle to muscle variation, measurements were normalized to the initial measurements. Results show that scattering coefficients decreased with time and the decay rate became slower after a certain period. The inset in Fig. 3(a) shows an extended measurement over 30 h on a separate animal and indicates that scattering coefficients eventually arrived at a steady value at ~10 h. This time is in good agreement with the conventionally recognized rigor time. Results measured at another wavelength of 853 nm are also shown in Fig. 3(a). The trend is similar when using data measured at other wavelengths.

It is generally accepted that during development of rigor mortis, temporary cross-bridges form and "break" between the actin and myosin filaments in the overlap area. Previous studies[9,10] have revealed that rigor-induced sarcomere changes lead to a reduced refractive index difference between the A-band and I-band, which results in a smaller scattering efficiency. When rigor mortis development is complete, myosin

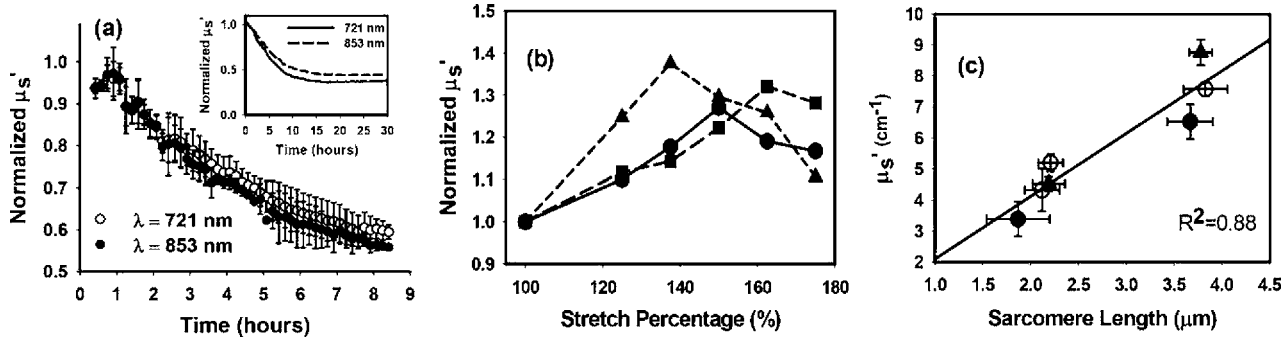

Fig. 3 (a) Changes in reduced scattering properties at 721 and 853 nm during the rigor mortis development in *sternomandibularis* muscles. The error bars indicate standard deviations. The inset shows an extended measurement up to 30 h postmortem. (b) Optical scattering properties measured at different muscle lengths during rigor mortis development. Results shown were from three different animals (muscles). (c) The relationship between sarcomere length and reduced scattering coefficients of postrigor *psoas major* muscles. The error bars indicate standard deviations.

S-1 heads and actins are bound together and the structural changes in the overlap area are arrested. Therefore, the measured reduced scattering coefficients reached a steady state. Curiously, the pH decrease normally associated with rigor mortis development also changes the refractive indices of the filaments and the sarcomere structures.[11] However, analyses of previous studies[9,12] reveal that reductions in pH should lead to an increase in reduced scattering coefficients. Thus these results suggest that the permanent formation of cross-bridges is the dominant effect in altering scattering coefficients. The other concern is the possible dehydration during the long postmortem process. However, due to the controlled experimental environment, moisture change is unlikely to play an important role in our experiment. We have measured the scattering coefficients of postrigor samples (with arrested sarcomere structure) for several hours and did not observe noticeable changes in results. In future development, a spectroscopy system with better sensitivity at 960~980 nm could be used to directly monitor the moisture change based on the water absorption band at ~970 nm.

To further investigate the effect of sarcomere changes on optical scattering, we measured the reduced scattering coefficients of whole prerigor *sternomandibularis* muscles while stretching the sample to different lengths along muscle fiber direction. Whole prerigor *sternomandibularis* muscles secured from beef carcasses immediately after slaughter were mounted on our experiment setup. Using our apparatus, muscles were stretched and restrained in such a manner that measurement location was held constant. Reduced scattering coefficients were calculated at each length. All measurements were concluded within 2 h of slaughter. Figure 3(b) shows results measured from three different animals (muscles). Measurements were normalized to the value at 100% length.

Figure 3(b) shows that starting from the nonstretched length, the reduced scattering coefficients increased with muscle stretch and reached a peak value at ~140% to 160% stretch. The μ_s' then decreased under further stretches. When muscle was stretched, sarcomere lengths increased. Based on previous studies, the intensity of high-order diffracted light increases with sarcomere length.[13] This is the equivalent of distributing more intensity to larger diffraction angles. Therefore, the scattering anisotropy g decreases and leads to the increase of the measured reduced scattering coefficient. Meanwhile, the decrease of the reduced scattering coefficient caused by rigor mortis (Fig. 3(a)) was small because of brevity of the test duration and its effect may have been overwhelmed by the sarcomere length increase induced effect. The observation that μ_s' decreased after a certain large stretch is likely due to the disruption of the sarcomere structures at excessive stretch, which reduces the light diffraction intensity[14] and counteracts the effects of increasing sarcomere length.

To further clarify the potential relationship between sarcomere length and tissue scattering, we measured the reduced scattering coefficients of postrigor *psoas major* samples of different sarcomere lengths as described in the methods section. Sarcomere length of each sample was determined using a modified protocol to that described elsewhere.[15] The results (Fig. 3(c)) clearly show that reduced scattering coefficients increased with sarcomere length. Each set of samples with the same symbol were obtained from the same animal, thus trends are consistent.

In summary, we conducted a series of *in vitro* studies that demonstrate the feasibility of monitoring sarcomere structural changes by measuring spatially resolved optical reflectance on whole muscle. A strong correlation existed between muscle optical scattering properties and sarcomere length. In addition, the results indicate that the optical scattering measurements can characterize structure changes during rigor mortis development. Our experimental observations are consistent with previous studies performed on single isolated muscle fibers.Of particular interest is the fact that reduced scattering coefficients were derived based on optical diffuse theories, which are inherently isotropic models for light migration in turbid media. Therefore, even though these results reveal certain underlying muscle sarcomere structures, it is possible that such derived scattering coefficients may not be quantitatively correct.

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