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Original Article

Validity and reliability of velocity measurements on ultrasonography using custom software with an optical-flow algorithm

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Abstract. [Purpose] The purposes of this study were: 1) to validate a commercial software program using an optical-flow algorithm to measure the velocity of muscle movement; and 2) to determine optimal image quality and the size and location of regions of interest. [Materials and Methods] First, a block of pork thigh muscle was pulled at 33 different constant velocities. Subsequently, an accelerometer, a high-velocity camera, and ultrasonography were used to obtain measurements, and an Echolizer software was used to determine ultrasound-based velocities. Finally, the impact of the location and size of the regions of interest and the brightness and contrast of the images was analyzed. [Results] The regression equation was expressed as y=1.150 × −0.071 with a determination coefficient of 0.996. The average absolute error of the software was 0.02 mm/s, and the average relative error was 0.20% of the actual velocity between 2.5 and 16.5 mm/s after the regression equation was applied to the measured data. The accuracy of measurement was reduced owing to the increased size of the regions of interest, which included poor image quality or a deeper zone. [Conclusion] Our method of measuring muscle velocity using a custom program showed high validity and reliability. It is necessary to use the regression equation in the program to improve accuracy. However, the validity of the method could be reduced if the regions of interest involve deep tissues or areas with poor visualization of the muscle bundles, or if the brightness and contrast of the image are set inaccurately.
Key words: Velocity, Ultrasound, Validation

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INTRODUCTION

A valid and reliable method of measuring gliding velocity on ultrasonographic images has not yet been established. When tissue adhesion limits gliding ability, it may contribute to a limited range of motion (ROM) or joint contracture[1, 2)], which can be clearly observed qualitatively on ultrasonography[3)]. Chen et al.[4)] manually measured sliding of the transverse abdominis by tracing the most lateral point of the deep fascia surrounding the transverse abdominis, and found that intra-rater reliability was 0.95. In a Japanese journal, Ichikawa et al.[5)] manually measured the distance between two arbitrary points on the deep fascia of the vastus lateralis during quasi-static knee flexion, and reported good intra-rater and inter-rater reliability (ICC 0.95–0.98) both in the superficial zone (i.e. subcutaneous tissue side) and the deep zone (i.e. vastus intermedius side) of the deep fascia. Both studies traced only one point on each ultrasound image. Tissue Velocity Imaging (TVI), an ultrasound-based technique with Doppler technology used to analyze cardiac function, can be used to measure the movement of the musculoskeletal system[6, 7)]. However, the measurement error of TVI ranged from 31% up to 313% when the phantom peak velocity was 0.03 cm/s[8)]. Moreover, the software for TVI was specially created for measuring cardiac motion and there is no program available to measure muscular movement. Therefore, there is an urgent need for a more valid and reliable quantifica-

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tion method of measuring muscle gliding including; (1) defining arbitrary regions of interest (ROI's) in the muscle; (2) tracing a greater number of measurement points in the ROI; (3) calculating the average or representative value; (4) quantifying the movement velocity of each ROI; (5) quantifying the relative velocity between ROI's; (6) visualization of the movement; and (7) comparing longitudinal data by matching the contours of bones to assure the ultrasound image observations are the same size. A highly specialized software program is required to achieve these needs.

Echolizer (GLAB Co., Inc., Japan) is a commercial software package that uses an optical-flow alorithm to measure the movement velocity of muscle bundles on ultrasound images. Using Echolizer software to determine the movement velocity of muscles and other soft tissues, the clinical outcomes of physical therapy or hydrorelease[9, 10] interventions could be quantified. Measurement errors can be eliminated by averaging the velocity of several measurement points within the region of interest (ROI). Abnormal values can be eliminated by including only those within 2.0 standard deviations of the velocity values. It is not expected that human factors would cause any measurement errors because ROI's are manually defined in a reproducible manner and velocity measurements are fully automated. Determining the optimal data acquisition method as well as the optimal analysis method such as the appropriate settings for the video frame rate, image qualities such as brightness and contrast, effects of the size and position of the ROI is expected to further improve the validity and reliability of Echolizer measurements. No studies to date have validated the optical-flow algorithm for velocity measurements of skeletal muscles and, therefore, this study would provide novel knowledge for musculoskeletal research community. The purposes of this study were to determine: (1) the validity and reliability of Echolizer software in measuring the velocity of the muscle bundles; and (2) the optimal image quality and the size and location of ROI's to optimize the validity.

The optical-flow algorithm calculates velocity of an object and present arrows to present the velocity and orientation of the object. The Ferneback method calculates movements of all pixels by comparing sequential images[11, 12]. This method can be used in automatic driving technology, which has an absolute error in collision time estimation within 0.5 seconds[13]. The relative error for blood flow velocity estimation was within 4%[14]. The average relative error for tendon motion tracking on ultrasound image sequences using the optical flow algorithm was less than 12.1%. Williamson et al.[15] reported that tracking changes in liver morphology on ultrasound images using the optical-flow algorithm demonstrated an accuracy (mean ± standard deviation) of 0.74 ± 1.03 mm. Tabata et al.[16] examined the accuracy of tagged magnetic resonance optical-flow velocity measurement and found that the relative error was less than 1%. Therefore, the validity and reliability of Echolizer are considered high.

The hypothesis of this study was that, in locations where the muscle bundle can be clearly seen on ultrasound images, the software would demonstrate high validity and reliability in measuring muscle bundle movement velocity. This software is expected to allow a wider range of measurements of any moving tissues without involving human errors in either the detection or tracking of the specific tissue being examined. To confirm or deny this hypothesis, we performed a validation study using pork meat. If a method of measuring muscle glide with high validity and reliability can be established, it may contribute to measuring the effectiveness of physical therapy interventions on soft tissue movement.

MATERIALS AND METHODS

This study is a cross-sectional study. Since the two pieces of pork meat used as materials were obtained at a local butcher shop, review of the study protocol by the ethical committee was not required under ethical guidelines for the use of animals in research.

Two pieces of pork thigh meat measuring 3 × 20 × 15 cm each were obtained at a local butcher shop. The pork meat was vacuum-packed and securely sealed using adhesive tape. Each vacuum-packed bag was sandwiched between two 28 × 20 cm wooden photo frames (wooden frame) and fixed by screws. The two frames were then stacked on top of each other. The inside of the bags and the layer between the two bags were filled with ultrasonic gel to ensure the muscle fibers of the pork meat would be visible on ultrasonography. On the experimental bench, the two meat bags in wooden frames were placed between two parallel wooden planks that formed rails for the wooden frames to glide between (Fig. 1a). A 1 kg weight was placed on the top meat to apply pressure that helped maintain close contact between the two bags of meat. A wooden block was attached to the bench in front of the lower meat frame in order to stabilize the meat and prevent wobbling during towing. The upper meat frame was connected by a wire to the universal testing machine (UTM) (AG-10TE, Shimadzu Corp., Japan). The wire load strength was 20 kg. A 1 kg weight was hung from the table by a wire connected to the opposite end of the upper meat frame in order to maintain constant tension between the UTM and the upper meat frame (Fig. 1b). To attach the ultrasonic probe (Linear Probe L11-3, Konica Minolta Inc., Japan), a rectangular hole was created in the workbench under the lower meat frame (Fig. 1c). The probe was inserted from the bottom and fixed using adhesive tape so that contact with the lower side of the meat bag was maintained. A high-velocity video (EX-FH25, Casio Computer Co., Ltd., Japan) was installed over the meat frames and an accelerometer was attached to the upper meat frame to monitor movement of the meat frames. A ruler was placed on the workbench next to the pork so that the scale of movement could be recorded by the high-velocity camera. Room temperature was set and maintained at 24 °C. Using the UTM, the upper meat was pulled at a constant velocity during each session; 33 sessions were performed at 33 different velocities ranging from 0.5 mm/s to 16.5 mm/s at 0.5 mm/s increments. Ultrasonic B-mode using an ultrasound imaging device (SONIMAGE HS1. Version 1.31, Konica Minolta, Inc., Japan) was used for imaging. A high-velocity video camera and an accelerometer were used to monitor the movement of

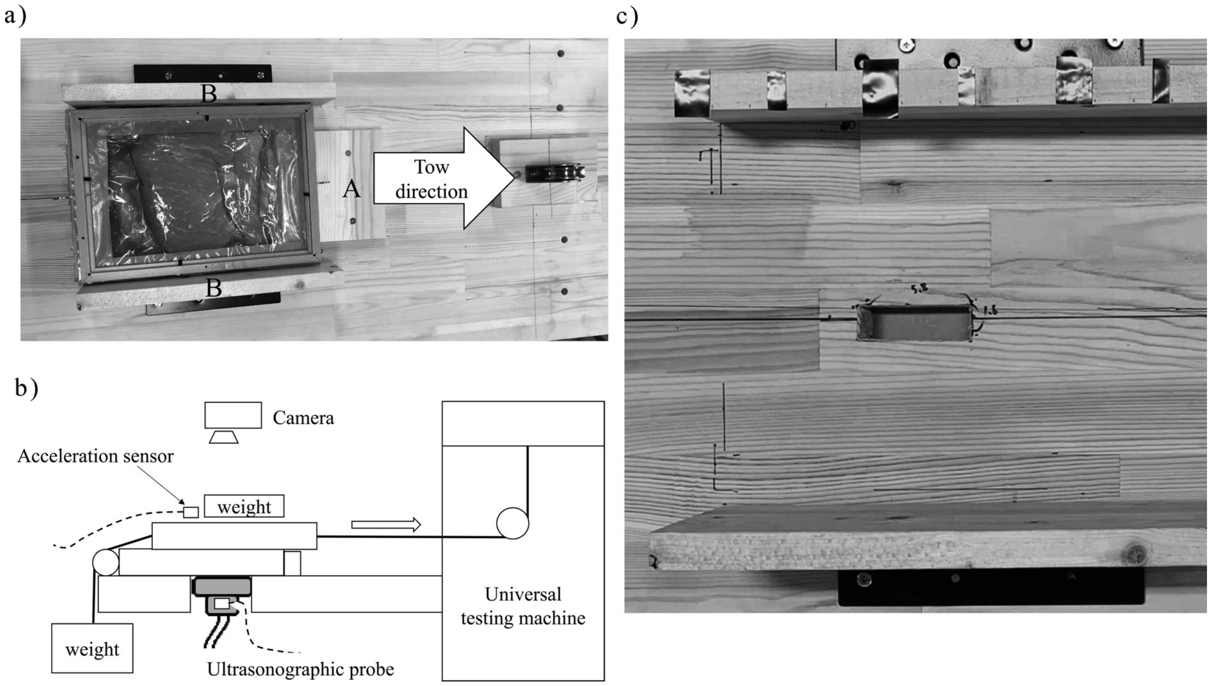

Fig. 1. Experimental setting.
a) The specimen (pork meat) was vacuum-packed and contained in a wooden frame. Two wooden frames were overlaid and the layer between the specimens was filled with ultrasonic gel. Hooks were attached proximally and distally to the upper wooden frame and then wires were attached to these hooks. A stopper (A) was attached to the base so that the lower wooden frame did not move during towing. Two wooden boards (B) were attached to the base so that the two wooden frames did not rotate or wobble during movement.
b) The wooden frame containing the specimen was placed on the base, and the meat was fixed to the base. The upper frame was connected to the universal tester attachment via a wire. A 1 kg weight was hung distally to maintain constant tension on the wire. The 1 kg weight was placed on the meat to maintain the approximation of the two frames. A high speed camera was positioned above the workbench and an acceleration sensor as well as a ruler were installed on the upper frame. An ultrasonic probe was positioned in a hole cut into the surface of the workbench in order to record movement of the specimens during measurements. The upper frame was pulled at 32 different velocities (ranging from 0.5 to 16.5 mm/s at increments of 0.5 mm/s).
c) A rectangle hole was created in the workbench to firmly hold the probe during measurements and an adhesive tape was used to stabilize the probe.

the wooden frames. The experiment was performed by a physical therapist (TY) with five years of experience in ultrasound imaging. Since the ultrasound probe was fixed in the wooden rectangle, it was not expected that there would be any effects of technical variations due to the investigator's experience.

Analyses were performed by the same investigator (TY) using Echolizer software with the Farneback method and the optical flow algorithm (Fig. 2). The Farneback method measures the movement of an object within grids on the ultrasound image without using the characteristic point (or edge) within the ROI. First, ultrasound movie files were imported into Echolizer program. The range of velocity for analyses was set between 0.0 and 30.0 mm/s for all variables including image quality (contrast and brightness), ROI size and location, and image frequency. The ROI's were defined using the grid overlaying the ultrasound image so that the ROI locations and sizes could be reproduced. The frequency of the image files to be analyzed were set at either frame-1 (30 Hz), frame-2 (15 Hz), or frame-3 (10 Hz), where frame-2 skipped one image and frame-3 skipped two images. Then, an analysis was performed, and mean velocity and mean orientation of the bundles in each ROI were obtained as a CSV format. Using data from the ROI located at the center of the ultrasonic image, regression analyses were performed to determine actual velocity using measured data from each velocity. The frequency settings of frame-1, 2 and 3 were respectively used for these analyses. The measured velocity was modified using the regression equation. Six ROI sizes and six locations were selected for analysis using the movie file obtained at 6.0 mm/s. The 6 sizes ranged from 6.25 to 900 mm^2 (2.5 × 2.5 mm, 5 × 5 mm, 10 × 10 mm, 15 × 15 mm, 20 × 20 mm, 30 × 30 mm (Fig. 3a). The six ROI locations included three in the superficial area and three in deeper portions of the meat. The location of each ROI was specified. The ROI size was set at 10 × 10 mm with intervals of 2.5 mm between the ROI's (Fig. 3b). Variables of the image quality involved 21 brightness and 21 contrast settings using a commercial software program (BeeCut, Apowersoft) using the movie file at 6.0 mm/s. The original image quality was 0 for brightness and 0 for contrast and −100 represented the lowest level of

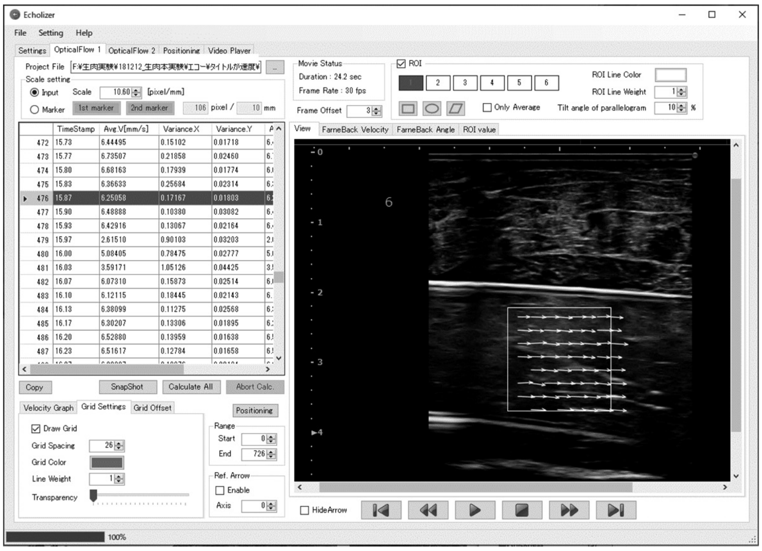

Fig. 2. Graphic interface of the Echolizer software.
Ultrasonographic images are imported as movie files, and up to six regions of interest (ROI's) can be defined. Velocity of muscle bundles is demonstrated by arrows, and the velocity and covariance are quantified and exported as a CSV format.

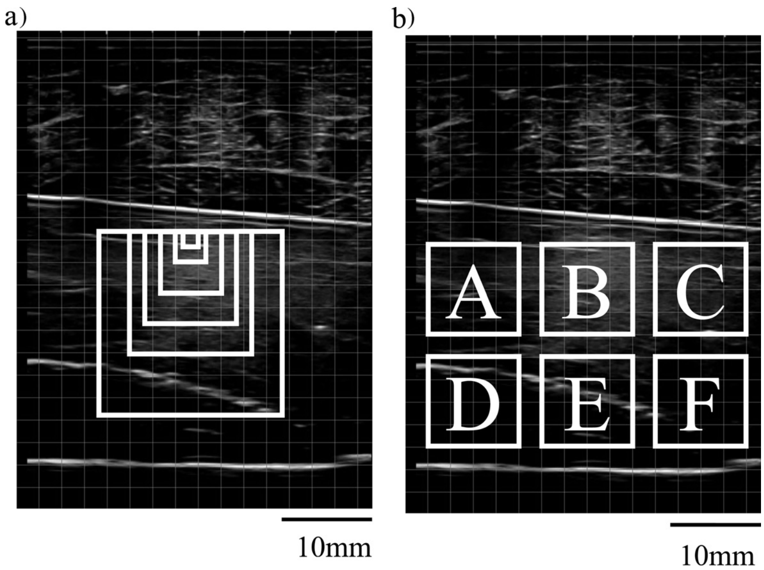

Fig. 3. Region of interest (ROI) settings.
a) Six ROI sizes were defined as 6. 25 mm^2 (2.5 mm long × 2.5 mm wide), 25 mm^2 (5 × 5 mm), 100 mm^2 (10 × 10 mm), 225 mm^2 (15 × 15 mm), 400 mm^2 (20 × 20 mm), 900 mm^2 (30 × 30 mm).
b) Six ROI locations with a consistent size (10 × 10 mm) were also defined.

brightness (darkest) and lowest level of contrast (Fig. 4a), while 100 represented the highest level of brightness and highest level of contrast (Fig. 4b). When the effects of brightness were analyzed, the contrast remained constant at 0. When the effects of contrast were analyzed, the brightness remained constant at 0. The ROI size remained constant at 100 mm^2 (10 ×10 mm) during both analyses.

A 120 Hz high-velocity camera and acceleration data confirmed whether the upper meat was being pulled at a constant velocity during the measurement and whether the probe remained still during towing. The movie from the high-velocity video was qualitatively analyzed to confirm that the movement of the wooden frame was constant. The accelerometer data was analyzed to confirm that the acceleration remained at 0 mm/s during towing.

SPSS statistics ver. 23 (manufactured by IBM SPSS) and G*Power 3.0 were used for statistical analyses. The significance level was set at α=0.05. Actual velocity and the measured data were used in the regression analysis to determine the accuracy

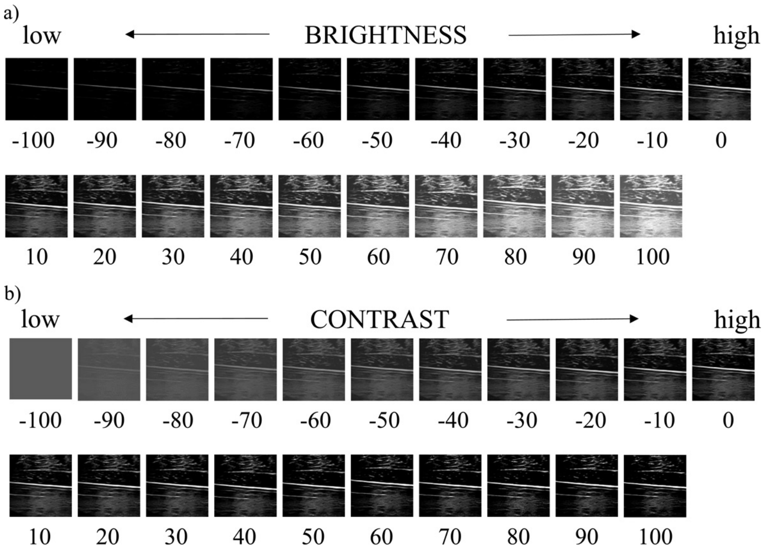

Fig. 4. Definitions of brightness.
a) The brightness of the original image was set at 0, with the lowest and highest brightness being −100 and 100, respectively.
b) The contrast of the original image was set at 0, with the lowest and highest contrast being −100 and 100, respectively.

and its reliable range in the velocity. Descriptive statistics were used for the effect of image quality, size and location of the ROI's, and frequency. A priori power analysis was performed and the required sample size was 26 with an Effect size (r) of 0.5, an α error probability of 0.05, and a Power (1-β prob) of 0.8.

RESULTS

The regression equation, the determination coefficient of the actual velocity and the measured data under three movie frequencies are shown below:

Frame-1 (30 Hz): $y=3.802x + 0.8984$, $R^2=0.166$, $p=0.019$

Frame-2 (15 Hz): $y=0.977x + 0.5939$, $R^2=0.990$, $p<0.001$

Frame-3 (10 Hz): $y=1.150x + 0.071$, $R^2=0.996$, $p<0.001$

The data in frame-3 involved a relative error of 12.1% between 2.5 and 16.5 mm/s (Fig. 5a). After regression equation was applied to the measured data, the absolute error was an average of 0.02 [95%CI: −0.11, 0.14] mm/s (Fig. 5b), and the relative error was 0.2 [−0.8, 1.2] % (Fig. 5c).

Effects of the ROI size were analyzed using six sizes ranging from 25 to 900 mm^2 on ultrasound images obtained at 6.0 mm/s (Table 1). The median velocity of the six ROI's was 6.06 (range: 4.02 to 6.13) mm/s. The minimum error was observed with a ROI of 225mm^2, in which the absolute and relative errors were 0.03 mm/s and 0.51%, respectively. The maximal error was observed with a ROI of 900 mm^2, in which the absolute and relative errors were −1.98 mm/s and 33.0%, respectively.

Effects of the ROI location were analyzed using six ROI locations on ultrasound images obtained at 6.0 mm/s (Table 2). ROI's A, B and C were located on the superficial area, while ROI's D, E and F were on the deeper area. The average velocity of the six ROI's was 4.07 [2.19, 5.95] mm/s. The relative errors for the ROI's B and C were 2.4 and −4.9%, respectively, whereas those for the ROI's A, D, E and F ranged from −25.4% to −79.3%. Note that the image quality of ROI A was not clear enough to show details of the muscle bundles compared with the image quality of ROI's B and C.

Effects of the brightness and contrast were examined at 6.0 mm/s using 21 levels of brightness and contrast, respectively. The median velocity was 5.77 (0.74 to 5.81) mm/s (Fig. 6, Table 3). The relative error exceeded 10% with a brightness of −50 or below, but remained within 5% at a brightness of −30 or above. The median velocity was 5.73 (−0.07 to 5.83) mm/s (Fig. 7, Table 4). The relative error exceeded 10% with a contrast of −60 or below, but remained within 5% with a contrast between −30 and 80.

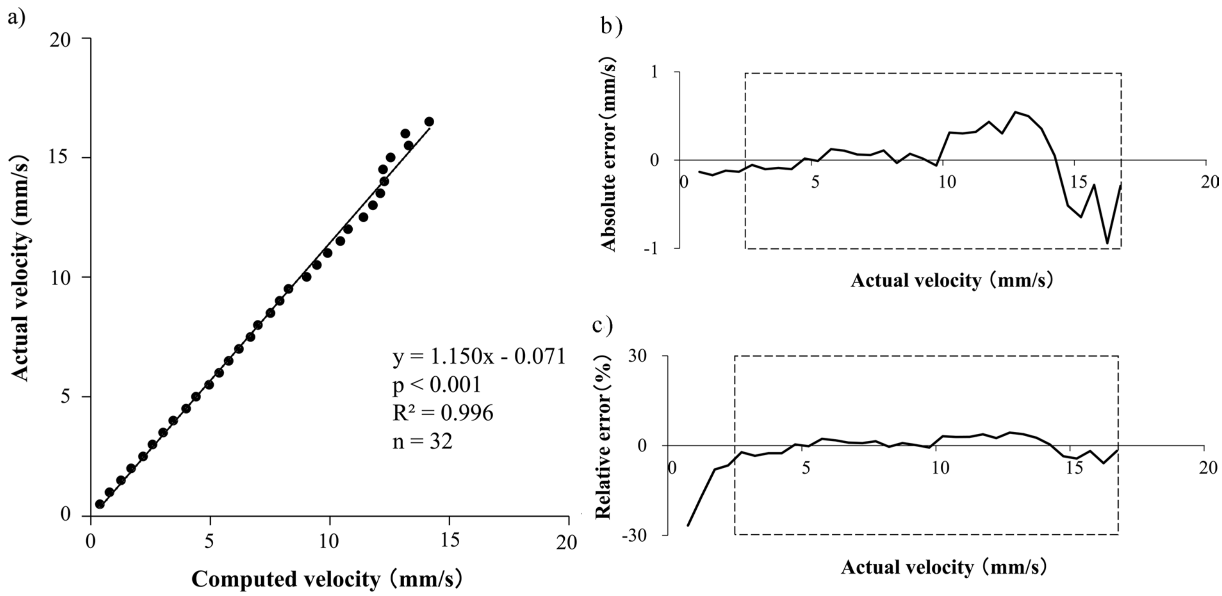

Fig. 5. Regression analysis of the computed velocity as an independent variable and actual velocity as the dependent variable.
a) The regression equation was y=1.150x−0.071, and R^2 was 0.996.
b) After regression analysis was applied to the computed data, the average [95%CI] of the absolute error was 0.02 [−0.11, 0.14] mm/s in the range between 2.5 to 16.5 mm/s (area within the dotted line).
c) After regression analysis was applied to the computed data, the average [95%CI] of the relative error was 0.2 [−0.8, 1.2]% in the range between 2.5 to 16.5 mm/s (area within the dotted line).

Table 1. Absolute and relative errors in different ROI sizes

ROI size (mm^2)	Actual velocity (mm/s)	Software calculation (mm/s)	Absolute error (mm/s)	Relative error (%)
6.25 (2.5 mm × 2.5 mm)	6.00	6.09	0.09	1.5
25 (5 mm × 5 mm)	6.00	6.13	0.13	2.1
100 (10 mm × 10 mm)	6.00	6.10	0.10	1.6
225 (15 mm × 15 mm)	6.00	6.03	0.03	0.5
400 (20 mm × 20 mm)	6.00	5.81	−0.19	−3.1
900 (30 mm × 30 mm)	6.00	4.02	−1.98	−33.0

Table 2. Absolute and relative errors in different ROI locations

Location	Actual velocity (mm/s)	Software (mm/s)	Absolute error (mm/s)	Relative error (%)
A	6.00	4.47	−1.53	−25.4
B	6.00	6.14	0.14	2.4
C	6.00	5.71	−0.29	−4.9
D	6.00	1.24	−4.76	−79.3
E	6.00	3.34	−2.66	−44.3
F	6.00	3.52	−2.48	−41.3

DISCUSSION

The purposes of this study were to determine: (1) the validity and reliability of Echolizer software in measuring the velocity of muscle bundles; and (2) the optimal image quality as well as ROI size and location to enhance the validity. The regression equation of y=1.150x + 0.071 with a determinant coefficient of R^2=0.996 shows an excellent fit. After this

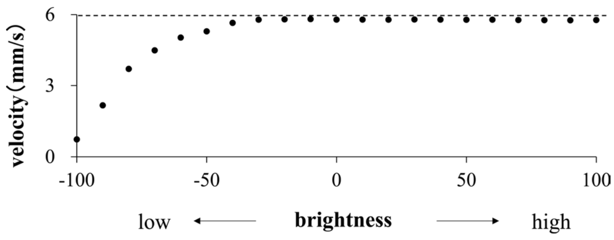

Fig. 6. Effects of brightness on the computed velocity.
The actual speed was 6.0 mm/s (horizontal line). The relative error exceeded 10% with a brightness of −50 or below, but was within 5% with a brightness of −30 or above.

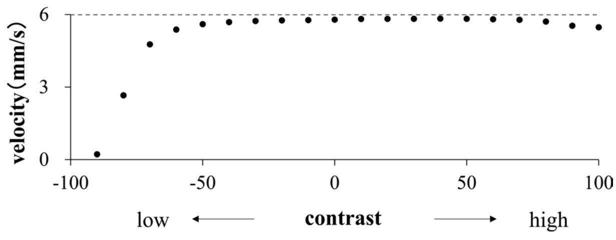

Fig. 7. Effects of contrast on the computed velocity.
The actual speed was 6.0 mm/s (horizontal line). The relative error exceeded 10% at a contrast of −60 or below, but was within 5% at a contrast between −30 and 80.

Table 3. Absolute and relative errors in velocity at different brightness levels

Brightness	Actual velocity (mm/s)	Software (mm/s)	Absolute error (mm/s)	Relative error (%)
−100	6.00	0.74	−5.26	−87.7
−90	6.00	2.16	−3.84	−64.0
−80	6.00	3.70	−2.30	−38.3
−70	6.00	4.49	−1.51	−25.2
−60	6.00	5.03	−0.97	−16.2
−50	6.00	5.29	−0.71	−11.8
−40	6.00	5.65	−0.35	−5.9
−30	6.00	5.78	−0.22	−3.6
−20	6.00	5.80	−0.20	−3.4
−10	6.00	5.81	−0.19	−3.2
0	6.00	5.79	−0.21	−3.6
10	6.00	5.79	−0.21	−3.6
20	6.00	5.78	−0.22	−3.6
30	6.00	5.79	−0.21	−3.4
40	6.00	5.79	−0.21	−3.6
50	6.00	5.78	−0.22	−3.6
60	6.00	5.78	−0.22	−3.6
70	6.00	5.77	−0.23	−3.8
80	6.00	5.76	−0.24	−3.9
90	6.00	5.76	−0.24	−4.0
100	6.00	5.77	−0.23	−3.9

equation was applied, the relative error was 0.20%. The measurement accuracy was decreased for deep ROI locations, ROI’s with low quality visualization of the muscle bundles, large ROI’s located in a deeper zone, images with low brightness and low or high contrast.

Regression analysis of the measured velocity using Echolizer program with the optical-flow algorithm and block matching method obtained a relative error of 0.2%, which was considered excellent. Although we utilized echo movies of 30 Hz, the best determinant coefficient of 0.996 was obtained at 10 Hz by skipping two frames in-between. A previous study showed that the average relative error of the speckle tracking algorithm with the block matching method was 1.3% in measuring the velocity of the Achilles tendon in pig cadavers[17]. In measurements using another optical-flow algorithms, Williamson et al.[15] reported that the accuracy (mean ± standard deviation) was 0.74 ± 1.03 mm, and Tabata et al.[16] reported a relative error of less than 1%. The current study showed an excellent accuracy of 0.2% after correction for detecting a velocity between 2.5 and 16.5 mm/s.

The ROI size and location may influence accuracy. A ROI size of 225 mm^2 produced the smallest relative error of 0.5%, whereas a size of 900 mm^2 produced the largest relative error of 33.0%. For sizes below 225 mm^2, the greater the ROI size, the smaller the relative error. This suggests that the greater number of muscle bundles visible in a larger ROI may be advantageous to achieving a higher accuracy. However, a ROI size of 400 mm^2 or larger demonstrated a lower accuracy because

Table 4. Absolute and relative errors in the velocity in different contrast levels

contrast	Actual velocity (mm/s)	Software (mm/s)	Absolute error (mm/s)	Relative error (%)
−100	6.00	−0.07	−6.07	−101.2
−90	6.00	0.22	−5.78	−96.3
−80	6.00	2.66	−3.34	−55.7
−70	6.00	4.76	−1.24	−20.6
−60	6.00	5.38	−0.62	−10.4
−50	6.00	5.60	−0.40	−6.7
−40	6.00	5.69	−0.31	−5.2
−30	6.00	5.73	−0.27	−4.5
−20	6.00	5.76	−0.24	−4.1
−10	6.00	5.77	−0.23	−3.9
0	6.00	5.79	−0.21	−3.6
10	6.00	5.81	−0.19	−3.1
20	6.00	5.82	−0.18	−3.0
30	6.00	5.82	−0.18	−3.0
40	6.00	5.83	−0.17	−2.9
50	6.00	5.82	−0.18	−3.0
60	6.00	5.80	−0.20	−3.3
70	6.00	5.78	−0.22	−3.7
80	6.00	5.71	−0.29	−4.9
90	6.00	5.54	−0.46	−7.7
100	6.00	5.47	−0.53	−8.8

the ROI's were located in the deeper zone and the muscle bundles could not be seen clearly. Regarding the ROI locations, the relative error at locations B and C were 2.4% and −4.9%, respectively, where the muscle bundles were clearly shown. In locations A, D, E and F, the muscle bundles were not clearly demonstrated and therefore, showed significantly lower accuracy with relative errors of −25.4% or greater. ROI locations in the deeper zone produced greater relative errors due to poor clarity of the muscle bundles. Although location A was in the superficial zone, the relative error was −25.4% because of the dark area on the left side of the image. Although the cause of this darkness was not identified, the poor clarity of the muscle bundles in the ROI caused a large relative error. The Farneback method used in Echolizer calculates the optical-flow of all pixels in a ROI8, 9. Therefore, it is reasonable that the clearness of the muscle bundles influences the measurement accuracy. Accordingly, higher accuracy of Echolizer program would be achieved by defining the ROI containing greater number of clear muscle bundles.

Effects of the brightness and contrast of the ultrasound image were verified. Good results with a relative error less than 5% were obtained when the brightness was set at −30 or greater and the contrast was set between −30 and 80. The velocity was underestimated when the brightness and contrast were lower than −30, at which clarity of the muscle bundles was visually inferior and the edges were more difficult to recognize. Visual transparency reduces the accuracy of the analysis[18–21]. Therefore, it is highly important that the original image quality of the ultrasonography should contain clear muscle bundles, which requires the examiners' experience in acquiring the ultrasonographic images. The important message from this result is that the image contrast nor brightness should not be changed to a large extent since the measurement accuracy is primarily determined by the quality of the original ultrasonographic images.

We made every effort to improve the internal validity of Echolizer measurement. The actual movement of the material during the testing was monitored by both an acceleration sensor and a high-speed camera in order to confirm that the specimen was pulled at a uniform velocity. Therefore, the movement velocity of the material is considered the true value. Sufficient statistical power for regression analysis was achieved by testing at 33 different velocities. This is the first study to validate a software program that quantifies muscle velocity without using anatomical landmarks.

There are several limitations. First, the material was neither human nor living. However, visual observation of pork meat and muscles of living humans were similar in our preliminary studies. The ultrasound image can detect the fascia such as tendons and intra-muscular tendons of the muscles and histological similarities between human muscles and pork meat have been reported. We carefully checked the appearance of the pork meat on the ultrasound images and found that the muscle fascicles appeared similar to those of humans. Furthermore, we used the graphic interface to confirm that the software could successfully detect the fascicles. Therefore, we believe the selected material was appropriate. Second, the material was not a living animal and this might have reduced the water content within the meat, which could have negatively impacted the accuracy. Third, the quality of the ultrasonographic images affects the accuracy of the velocity measurement. Therefore, we should be cautious in selecting high-quality ultrasonographic images obtained by an experienced researcher or technician. Fourth, there is an effect of tissue deformation in the longitudinal direction of the muscle bundles. We used a wooden frame to contain the specimen and longitudinal muscle elongation was considered minimal. Fifth, the orientation of the probe was set parallel to the direction of towing in this study, which limited our ability to validate the software when the direction of muscle movements is not parallel to the orientation of the probe[22].

To conclude, the optical-flow algorithm had a relative error of 12.1% in the measurement of muscle bundle movements. After correction using the regression equation, the relative error of Echolizer program was within 0.20% between 2.5 and 16.5 mm/s. The validity can be reduced if the ROI's are set to include deep tissue or areas with poor visualization of the muscle bundles, or image brightness and contrast are set too low or too high. Echolizer program does not require anatomical landmarks on ultrasonic images. Therefore, this study provides novel information on the quantification of tissue movements using ultrasonic images. Further studies should confirm its validity in living human tissues and this software is expected to reveal local gliding properties *in vivo*, which would allow therapists to quantify the effectiveness of physical therapy interventions on various soft tissues.

Conflict of interest

Echolizer program was temporarily licensed by GLAB Co., Ltd.

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