The use of a Lucas-Kanade based template tracking algorithm to examine in vivo tendon excursion during voluntary contraction using ultrasonography

Kiros KaramanidisA,B, Artemis TravlouA,C, Peter KraussA, Uwe JaekelD

A Institute of Movement and Sport Gerontology, German Sport University Cologne, Am Sportpark Müngersdorf 6, 50933 Cologne, Germany

B Institute of Biomechanics and Orthopaedics, German Sport University Cologne, Am Sportpark Müngersdorf 6, 50933 Cologne, Germany

C National and Kapodistrian University of Athens, School of Science Department of Physics, 30 Panepistimiou Street, 10679 Athen, Greece

D Faculty of Mathematics and Technology, University of Applied Sciences, Koblenz, Joseph-Rovan-Allee 2, 53424 Remagen, Germany

Corresponding Author:

Kiros Karamanidis

Institute of Movement and Sport Gerontology, German Sport University Cologne

Am Sportpark Müngersdorf 6, 50933 Cologne, Germany

Tel.: +49 (0)221 49826144

Fax: +49 (0)221 49826143

Email: karamanidis@dshs-koeln.de

Abstract

Ultrasound imaging can be used to study tendon movement during muscle contraction to estimate tendon force-length relationship in vivo. Traditionally, such tendon displacement measurements are conducted manually (time consuming and subjective). Here we evaluated a Lucas-Kanade based tracking algorithm with an optic flow extension that accounts for tendon movement characteristics between consecutive frames of an ultrasound image sequence. Eleven subjects performed 12 voluntary isometric plantarflexion contractions on a dynamometer. Simultaneously, the gastrocnemius medialis tendon was visualized via ultrasonography. Tendon displacement was estimated manually and by using two different automatic tracking algorithms. Maximal tendon elongation (manual: 17.9±0.3mm; automatic: 17.0±0.3mm) and tendon stiffness (209±4N/mm; 218±5N/mm) generated by the developed algorithm correlated with the manual method ($0.87 \leq R \leq 0.91$) with no differences between methods. Our results suggest that optical flow methods can potentially be used for automatic estimation of tendon movement during contraction in ultrasound images, which is further improved by adding a penalty function.

Key words: Ultrasound, optical flow, automatic tracking, Achilles tendon, voluntary contraction, Lucas-Kanade

Introduction

Analysis of human tendon length changes from ultrasound (US) images during maximal voluntary muscular contraction performed on a dynamometer is widely used, and has become highly popular, to assess the in vivo force-length-relationship of the tendon (Maganaris and Paul 2000; Arampatzis et al. 2005; Reeves et al. 2005). The benefits of the method are that it is non-invasive, affordable, easily applied and it tracks a quantity that is proposed as a surrogate measure of tendon mechanical properties. The application of the US method synchronously with force measurements has provided relevant information with respect to tendon injury, and tendon adaptive changes due to aging, disuse and various physical exercise interventions (Reeves et al. 2003, 2005; Arya and Kulig 2010; Karamanidis and Arampatzis 2007; Arampatzis, et al. 2007).

Tendon length changes by US during muscular contraction is usually estimated by choosing a tissue landmark (e.g. myotendinous junction) and manually digitizing that landmark frame by frame from rest until maximal tendon force (Arampatzis et al. 2005; Arya and Kulig 2010). Manual tracking, however, may be time consuming and requires a lot of experience. An automated method for tracking tendon length changes from US images during voluntary contractions on dynamometric devices would provide a time-efficient means for assessing tendon elongation and the force-length relationship. Moreover, if tendon elongation could be accurately assessed during contraction, instead of post-measurement by manually digitizing a tissue landmark frame by frame, an immediate assessment of tendon mechanical properties would be possible. Once examined for its accuracy, such an analysis method would provide a time-efficient means for assessing human tendon stiffness *in vivo*, and could have significant applications in clinical and scientific settings.

Several attempts have recently been made to implement automated tracking by determination of the optical flow between successive US images (Lee et al. 2008; Korstanje et al. 2010; Pearson et al. 2013; Kim et al. 2011). Optical flow is defined as the distribution of apparent velocities for individual pixels between two images (Horn and Schunck 1981). The Horn-Schunck algorithm is a global method determining the optical flow over the whole image frame. In our numerical experiments, we found that a regularization term controlling the smoothness leads to a considerable lag of the integrated optical flow behind manual tracking. However, a number of approaches have previously been taken in an attempt to automatically track tendon displacement (Lee et al. 2008; Korstanje et al. 2010; Pearson et al. 2013; Kim et al. 2011). Of these, only one study examined voluntary contractions and compared an automated tracking method using with manual measures of highly loaded in vivo tendon excursions (Pearson et al. 2013), revealing difference in the maximal elongation of the tendon between methods of $\leq$ 0.81 mm for a mean displacement value of about 16.5 mm. We base our approach on minimization of the sum of the squared differences between a template region and a warped image. This approach differs from Pearson et al. (2013) who used normalized cross correlations for automatic tracking of *in vivo* displacement of the tendon. While we allow linear-affine deformations such as rotational, shearing or scaling transformations of the matched regions, it seems that Pearson et al. used direct cross correlations of the matched regions. Allowing deformations makes our method suitable for the analysis of rather large frame-to-frame displacements and deformations, as well as for lower framerates. In that study (Pearson et al. 2013), only one subject was examined, thereby neglecting differences in image quality across subjects that will affect the ability of the algorithm to track the tendon accurately during loading. In addition, none appear to have examined whether the estimation of optical flow on an US video can be improved by adjusting the algorithm to the tendons highly coherent movement during loading.

Therefore, we aimed to develop a Lucas-Kanade optical flow based template tracking algorithm (Lucas and Kanade 1981) that eliminates any unwanted jumps in the tracking of the gastrocnemius medialis tendon (GM_{tendon}) elongation during maximal voluntary isometric ankle plantar flexion contractions (MVIP) on a dynamometer. In addition, we aimed to compare, in vivo, our discussed modified automated method with both the established manual method and the automated tracking method proposed by Schreiber (2007) in wide range of different US videos to examine the accuracy of our current tracking algorithm during MVIP. As direct measurement of human tendon elongation is not possible in vivo, automated tracking was compared with manual tracking in living subjects using the same US image sequences. We hypothesized that the developed tracking algorithm that takes into account the characteristics of the movement of the GM_{tendon} in US videos during MVIP will generate a higher agreement than the initial Lucas-Kanade based optical flow algorithm proposed by Schreiber (2007) when compared to the values assessed by the manual tracking method.

Materials and Methods

Experimental setup and joint kinetics analysis

Eleven healthy young male subjects (means and SD; age: 28 ±6 yrs.; body height: 179 ± 4cm; body mass: 75.5 ± 7.8kg) participated in the study. Approval was obtained from the university's committee for the protection of human subjects and informed consent was given by all subjects.

After warming up (combination of hopping and stretching for about 5 minutes to precondition the tendon), the subjects were seated on a custom built dynamometer with the shank perpendicular to the foot and the knee fully extended (neutral position; see Fig. 1). A custom made harness built from ski bindings was applied around the foot and the dynamometer foot plate to reduce any joint motion during contraction. All subjects had to perform 6 MVIP contractions during two different sessions on the dynamometer, using either the left or the right leg. The instructions given to the subjects were to produce maximal isometric force ramp contractions, gradually increasing the plantarflexion effort over 3-5 seconds (loading) and to hold the achieved moment about 2-3 seconds similar to methods reported in the literature (Arampatzis, et al. 2007; Karamanidis and Arampatzis 2007).

Fig. 1

The resultant moments at the ankle joint were calculated using inverse dynamics and the compensation of moments due to gravitational and compression forces was done for all subjects before each plantarflexion contraction (Arampatzis, et al. 2007; Karamanidis and Arampatzis 2007). To calculate the lever arm of the ground reaction force acting about the ankle joint during plantarflexion contractions, the point of force application under the foot was assessed via dynamometry (see Fig. 1). In order to do so, the reaction forces under the foot during contraction were determined by three strain gauge load cells fixed at predefined distances on the foot plate (100Hz; Fig 1). The axis of rotation of the ankle joint was defined by the midpoint of the line connecting both malleoli. Eight light-emitting diodes (LEDs) were used as active markers to examine kinematics (Fig. 1). Four active markers were placed on the lower extremity (head of the fibula, malleolus lateralis, malleolus medialis and calcaneus) and four markers were fixed on the force plate at predefined locations. A motion capture system consisting of two digital high-speed cameras (Basler, Germany, 15Hz) was used to record the markers. The 2D trajectories of the markers were automatically tracked frame by frame via a custom-made algorithm in MATLAB (The Mathworks, Inc, Massachusetts, U.S.A., ver. R2010b). Due to the slow limb motion during such isometric voluntary ramp contractions, kinematic data were collected with a relatively low sampling frequency aimed to further shorten the amount of post processing time duration by our developed automatic marker tracking algorithm.

Fig. 2

The elongation of the GM myotendinous junction during contraction (see Fig. 2) was visually reproduced using a 7.5 MHz linear array US probe (fixed linear array frequency) and stored on the US device at 73Hz (Aloka α7, Tokyo, Japan). The probe was fixed at the myotendinous junction in a longitudinal direction according to the literature (Karamanidis et al. 2014). The GM myotendinous junction and hence, most proximal part of the GM tendon, which served as an anatomical marker, was identified for each individual before probe fixation by scanning the triceps surae muscle-tendon unit in the transversal plane. This procedure assured correct positioning of the probe for all subjects. Before probe fixation, an echo-absorptive marker was attached on the skin to act as a fixed reference from which manual and automatic measures of tendon elongation could be made, similar to previous works (Arampatzis et al. 2007; Karamanidis et al. 2014). From each subject, 12 US videos during MVIP were recorded, leading to a total of 132 US videos. In order to synchronize the different signals, two LEDs, a transistor-transistor logic (TTL) signal and an optical trigger on the US were used. All trigger signals were automatically identified using a custom-build semi-automatic analysis software in MATLAB. As a result, real-time synchronization of all signals was possible. The tracking of the length changes of the GM_{tendon} of all 132 US videos during the loading phase was performed both manually as well as by using two different automatic tracking algorithms: the Schreiber (2007) method and the current modified algorithm.

The start of the tendon tracking procedures were defined as when AT force was zero and ended when maximal tendon force was reached. AT force was calculated by dividing the ankle joint moment by the AT moment arm. The tendon moment arm was estimated for each individual by the perpendicular distance from the ankle joint centre of rotation (i.e. axis through the inferior tip of the medial and lateral malleoli) to the AT according to the method proposed by Scholz et al. (2008). Concerning tendon stiffness assessment for each tracking method, we used the method described previously (Karamanidis et al. 2014). Briefly, tendon elongation due to the inevitable ankle joint rotation during contraction (Magnusson et al. 2001) was calculated using the tendon excursion method (An et al. 1983; Maganaris 2000) by multiplying the estimated moment arm with the ankle joint angular rotation during contraction. In this way, the actual tendon elongation due to the exerted tendon force could be estimated. The stiffness of the tendon was calculated as the ratio of the increase in the calculated tendon force and the increase in the tendon elongation from 50 to 100% of the maximum tendon force (Karamanidis et al. 2014). Because the synchronization of all signals and the AT force calculation were accomplished in real-time, it was possible to perform the automatic US tracking procedure immediately after each measurement.

Manual tracking

For the manual tendon tracking a custom image data processing software was developed in MATLAB. The investigator marked a muscle fascicle in every frame of the recorded US video at the intersection with the bottom aponeurosis close to the GM myotendinous junction and digitized the US videos frame by frame from rest until maximal tendon force (Fig. 2). This lead to one set of manually tracked data for each of the 132 US videos. All manual tracking analyses were performed by one highly experienced investigator, and the tracked landmarks on the US videos were checked again by two further investigators for each video frame by frame, who were blind to the previous results. This was done in order to check all manual digitized landmarks as carefully as possible. Scaling in pixels per millimeter was assessed via MATLAB (2014a) software using the known depth and width of field in the US images (depth: 1 mm = 11.29 pixels or 1 pixel = 0.088mm; width: 1 mm = 10.67 pixels or 1 pixel = 0.094mm; US frequency: 7.5 MHz) as a calibration factor in the automated and manual tracking program to ensure equivalent pixel-to-millimeter ratios for all three tracking procedures.

Schreiber's Lucas-Kanade optical flow tracking algorithm

Each US video was also automatically tracked using a Lucas-Kanade (Lucas and Kanade 1981) based template tracking algorithm provided by Schreiber (2007) denoted here as the Schreiber algorithm. Using the notations of Baker and Matthews (2004), let $I_n(x)$ stand for the n^{th} image in a given video sequence, here $\mathbf{x} = (x_1, x_2)$ are the pixel coordinates and n = 0,1,2,.

. . is the frame number. A subregion of the initial frame $I_0(x)$ is extracted and becomes the template $T(\mathbf{x})$. Let $\mathbf{W}(\mathbf{x};\mathbf{p})$ denote the parameterized set of allowed deformations of the template, where $\mathbf{p} = (p_1, . . . ,p_k)^T$ is a vector of parameters. The warp $\mathbf{W}(\mathbf{x};\mathbf{p})$ takes the pixel $\mathbf{x}$ in the coordinate frame of the template $T(\mathbf{x})$ and maps it to a sub-pixel location, $\mathbf{W}(\mathbf{x};\mathbf{p})$, in the coordinate frame of the image $I_n(\mathbf{x})$. Lastly we denote the robust weights per pixel, used for tracking the template $T_1(\mathbf{x}) = I_0(\mathbf{x})$ in image $I_n(\mathbf{x})$, by $\omega_n(\mathbf{x})$.

The equation of the warp can be anything from a very simple translation $\mathbf{W}(\mathbf{x};\mathbf{p})= (x_1 + p_1, x_2 + p_2)^T$, if we have a planar non rotating object moving, to a complicated affine or even non-linear transformation.

For a realistic map of the 3D movement of tendon we restrict ourselves to a set of affine warps:

$$\mathbf{W}(\mathbf{x};\mathbf{p}) = \begin{pmatrix} (1+p_1)x + p_3 y + p_5 \\ p_2 x + (1+p_4)y + p_6 \end{pmatrix} = \begin{pmatrix} 1+p_1 & p_3 & p_5 \\ p_2 & 1+p_4 & p_6 \end{pmatrix} \begin{pmatrix} \mathrm{x} \\ \mathrm{y} \\ 1 \end{pmatrix} \qquad \text{(Eq.1)}$$

This parameterizes all possible linear-affine 2D transformations such as translations (characterized by the parameters p_5, p_6), rotations, shear and scaling transformations, and to some extent can handle also the US specific problem of continuous 3D structures that enter or leave the observed planar cross-section. For instance, the intersection of a three-dimensional ball entering or leaving the plane would appear as a growing or shrinking circle that is locally well described by an isotropic scaling transformation.

The only requirement for the set of warps is that they are differentiable with respect to the warp parameters. Schreiber (2007) introduced an algorithm as an extension to the inverse compositional algorithm that uses a fixed template. The goal of this was to find the best match to the template in the subsequent frame, and update the template in every step. The initial function that has to be minimized is:

$$\sum_{x}[I(\mathbf{W}(\mathbf{x};\mathbf{p}))-T(x)]^2 \qquad \text{(Eq.2)}$$

where minimization of the above expression is performed with respect to $p=(p_1,\dots,p_6)$, and the sum is performed over all the pixels of the template.

After a 1st order Taylor expansion on $I(\mathbf{W}(\mathbf{x};\mathbf{p})+\Delta\mathbf{p})$, and the introduction of robust weights, the least squares solution is:

$$\Delta\mathbf{p}=H_s^{-1}\sum_{x\in T}\omega_n(\mathbf{x})\left[\nabla T\frac{\partial\mathbf{W}}{\partial\mathbf{p}}\right][I_n(\mathbf{W}(\mathbf{x};\mathbf{p}))-T(\mathbf{x})] \qquad \text{(Eq.3)}$$

and the Hessian:

$$H_s=\sum_{\mathrm{x}}\omega_n(\boldsymbol{x})\left[\nabla\mathrm{T}\frac{\partial\mathbf{W}}{\partial\mathbf{p}}\right]^T\left[\nabla\mathrm{T}\frac{\partial\mathbf{W}}{\partial\mathbf{p}}\right] \qquad \text{(Eq.4)}$$

The robust weights are fixed, so the Hessian can be pre-computed.

Schreiber (2007) also uses a cumulative error function:

$$E_{n+1}(\mathbf{x})=(1-a)\cdot E_n(\mathbf{x})+a\cdot f([I_n(\mathbf{W}(\mathbf{x};\mathbf{p}))-T_1(\mathbf{x})]) \qquad \text{(Eq.5)}$$

where $a,$ is an adaption rate parameter with a typical value of 0.1.

After calculating the cumulative error function, the robust weight are updated as $\omega_{n-1}(\mathbf{x})=\eta(E_{n-1}(\mathbf{x}))$ where η is a robust estimator. We use the robust extension of the Lukas-Kanande method by Schreiber (2007) as reference method for a comparison with our method described below.

Current modified Lucas-Kanade optical flow tracking algorithm

The main obstacles of the automated tracking in US videos are the noise and the tissue irregularities that frequently lead to clearly non-physiological jumps of the matched region between two frames, since spurious correlations in the speckle noise patterns may dominate over the real information. However, the motion of tendons during active contraction is always continuous, and allowing big jumps can only lead to errors in tracking. In order to overcome this problem, we introduced a penalty function that effectively confines the motion of the matched regions between two frames to physiologically accessible velocities.

For the current work, we have chosen to track the US images with a Lucas-Kanade based template tracking algorithm. It is based on Schreiber's (2007) algorithm, with the addition of a jump penalty function. In order to penalize jumps over many pixels, a hyperbolic tangent function was inserted. The hyperbolic tangent function is differentiable, and at the same time can perform a penalization. The penalty function took the following form:

$$g(p) = \frac{\lambda}{2}\left(\tanh\left(\frac{p-d}{h}\right) + 1\right) \qquad \text{(Eq.6)}$$

where $p = \|\mathbf{p}\| := \sqrt{p_5^2 + p_6^2}$ is the size of the translation vector $(p_5, p_6)^T$ in pixels. The parameter d can be interpreted as a soft threshold for the acceptable jump size (in pixels), while h is a width parameter controlling the width over which the penalty function varies from negligible to large values in the vicinity of d. After testing the method on several US video formats and qualities, the parameters were set to $\lambda = 7, h = \mathrm{d} = 5$. With the choice $\lambda = 0$, the penalty term is switched off, and the method reduces to the classical Lucas-Kanade method. The parameter h controls the smoothness of the threshold. The larger the values of h, the smoother is the transition from 0 to the maximum value $g(\infty) = \lambda$. For $h \to 0$, the threshold becomes a step function.

Following the same steps as in the original Lucas-Kanade tracking algorithm, we want to minimize the expression:

$$\sum_x [I(\mathbf{W}(\mathbf{x};\mathbf{p}+\Delta\mathbf{p})) - T(\mathbf{x})]^2 + [g(\|\mathbf{p}+\Delta\mathbf{p}\|)]^2 \qquad \text{(Eq.7)}$$

with respect to $\Delta\mathbf{p}$, and then update the parameters $\mathbf{p}$ as $\mathbf{p} + \Delta\mathbf{p}$ iteratively.

After performing a first order Taylor expansion the expression to be minimized becomes

$$\sum_{x}\left[I\big(\mathbf{W}(\mathbf{x};\mathbf{p})\big) + \nabla I\frac{\partial\mathbf{W}}{\partial\mathbf{p}}\Delta\mathbf{p} - T(\mathbf{x})\right]^2 + \left[g(\|\mathbf{p}\|) + \frac{\partial g}{\partial\mathbf{p}}\Delta\mathbf{p}\right]^2 \qquad \text{(Eq.8)}$$

Following Hager and Belhumeur (1998), it is assumed that the current estimates of the parameters are approximately correct:

$I(W(x;p)) \approx T(x)$ which after using the chain rules becomes: $\nabla I\frac{\partial\mathbf{W}}{\partial\mathbf{x}} \approx \nabla T$.

That turns the previous expression to

$$\sum_{x}\left[I\big(\mathbf{W}(\mathbf{x};\mathbf{p})\big) + \nabla T\left(\frac{\partial\mathbf{W}}{\partial\mathbf{x}}\right)^{-1}\frac{\partial\mathbf{W}}{\partial\mathbf{p}}\Delta\mathbf{p} - T(x)\right]^2 + \left[g(\|\mathbf{p}\|) + \frac{\partial g}{\partial\mathbf{p}}\Delta\mathbf{p}\right]^2 \qquad \text{(Eq.9)}$$

where:

$$\left(\frac{\partial\mathbf{W}}{\partial\mathbf{x}}\right)^{-1} = \begin{pmatrix} 1+p_1 & p_3 - 1 \\ p_2 & 1+p_4 \end{pmatrix}^{-1} = \frac{1}{(1+p_1)(1+p_4) - p_2p_3}\begin{pmatrix} 1+p_4 & -p_3 \\ -p_2 & 1+p_1 \end{pmatrix} \qquad \text{(Eq.10)}$$

and

$$\left(\frac{\partial\mathbf{W}}{\partial\mathbf{x}}\right)^{-1}\frac{\partial\mathbf{W}}{\partial\mathbf{p}} = \frac{1}{(1+p_1)(1+p_4) - p_2p_3}\begin{pmatrix} x & 0 & y & 0 & 1 & 0 \\ 0 & x & 0 & y & 0 & 1 \end{pmatrix} \times$$

$$\times\begin{pmatrix} 1+p_4 & -p_3 & 0 & 0 & 0 & 0 \\ -p_2 & 1+p_1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1+p_4 & -p_3 & 0 & 0 \\ 0 & 0 & -p_2 & 1+p_1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1+p_4 & -p_3 \\ 0 & 0 & 0 & 0 & -p_2 & 1+p_1 \end{pmatrix} = \Gamma(\mathbf{x})\Sigma(\mathbf{x}) \qquad \text{(Eq.11)}$$

The partial derivative of the expression in Eq. (9) with respect to $\Delta\mathbf{p}$ is:

$$2\sum_{x}[\nabla T\Gamma(\mathbf{x})\Sigma(\mathbf{x})]^{T}\left[I\big(\mathbf{W}(\mathbf{x};\mathbf{p})\big)+\nabla T\Gamma(\mathbf{x})\Sigma(\mathbf{x})\Delta p-T(\mathbf{x})\right] + 2[\frac{\partial g}{\partial \mathbf{p}}]^{T}\left[g(\|\mathbf{p}\|)+\frac{\partial g}{\partial \mathbf{p}}\Delta\mathbf{p}\right] \tag{Eq.12}$$

Setting the previous expression equal to zero and solving for $\Delta\mathbf{p}$, gives us the minimum $\Delta\mathbf{p}$.

$$\Delta\mathbf{p}=-\left(\sum_{x}[\nabla T\Gamma(\mathbf{x})\Sigma(\mathbf{x})]^{T}[\nabla T\Gamma(\mathbf{x})\Sigma(\mathbf{x})]+[\frac{\partial g}{\partial \mathbf{p}}]^{T}[\frac{\partial g}{\partial \mathbf{p}}]\right)^{-1} \times\left(\sum_{x}[\nabla T\Gamma(\mathbf{x})\Sigma(\mathbf{x})]^{T}\left[T(\mathbf{x})-I\big(\mathbf{W}(\mathbf{x};\mathbf{p})\big)\right]+[\frac{\partial g}{\partial \mathbf{p}}]^{T}g\right) \tag{Eq.13}$$

This leads to the following algorithm steps:

Pre-compute:

1. Evaluate $[\frac{\partial g}{\partial \mathbf{p}}]^{T}\left[\frac{\partial g}{\partial \mathbf{p}}\right],[\frac{\partial g}{\partial \mathbf{p}}]^{T}g,\nabla T.$
2. Evaluate $\Gamma(\mathbf{x})\Sigma(\mathbf{x})$
3. Compute the matrices $[\nabla T\Gamma(\mathbf{x})\Sigma(\mathbf{x})],[\nabla T\Gamma(\mathbf{x})\Sigma(\mathbf{x})]^{T}$
4. Compute the matrix $\left(\sum_{x}[\nabla T\Gamma(\mathbf{x})\Sigma(\mathbf{x})]^{T}[\nabla T\Gamma(\mathbf{x})\Sigma(\mathbf{x})]+[\frac{\partial g}{\partial \mathbf{p}}\nabla g]^{T}[\frac{\partial g}{\partial \mathrm{p}}]\right)^{-1}$

Iterate:

5. Warp I, with $\mathbf{W}(\mathbf{x};\mathbf{p})$ to compute $I\big(\mathbf{W}(\mathbf{x};\mathbf{p})\big)$
6. Compute the error image $T(x)-I\big(\mathbf{W}(\mathbf{x};\mathbf{p})\big)$
7. Compute $\left(\sum_{x}[\nabla T\Gamma(\mathbf{x})\Sigma(\mathbf{x})]^{T}\left[T(\mathbf{x})-I\big(\mathbf{W}(\mathbf{x};\mathbf{p})\big)\right]+[\frac{\partial g}{\partial \mathbf{p}}]^{T}g\right)$
8. Compute

$$\Delta\mathbf{p} = -\left(\sum_{x}[\nabla T\Gamma(\mathbf{x})\Sigma(\mathbf{x})]^T[\nabla T\Gamma(\mathbf{x})\Sigma(\mathbf{x})] + [\frac{\partial g}{\partial \mathbf{p}}]^T[\frac{\partial g}{\partial \mathbf{p}}]\right)^{-1}$$
$$\times\left(\sum_{x}[\nabla T\Gamma(\mathbf{x})\Sigma(\mathbf{x})]^T\left[T(\mathbf{x}) - I\big(\mathbf{W}(\mathbf{x};\mathbf{p})\big)\right] + [\frac{\partial g}{\partial \mathbf{p}}]^T g\right) \quad \text{(Eq.14)}$$

9. Update $\mathbf{p} \leftarrow \mathbf{p} + \Delta\mathbf{p}$

All automatic tracking algorithms were implemented in MATLAB, along with a Graphic-User-Interface.

Statistics

In total, 132 different US videos of the GM_{tendon} during the loading phase of a MVIP were recorded and analyzed using the three different tracking methods: manual tracking that we considered as our gold standard, and automatic tracking once with the earlier Schreiber (2007) Lucas-Kanade optical flow template tracking algorithm, and once with the modified Lucas-Kanade based algorithm developed for the purposes of this work. The entire curve of the excursion of the GM_{tendon} during the loading phase, from rest until maximal tendon force, was considered for the comparison between the tracking methods. As a consequence, the same start and end US frame was used in each video for all three tracking methods. To determine the differences in absolute value between the three methods and to compare the entire curve of the excursion of the tendon, from rest until maximal tendon force, the root mean square error (RMSE) was used. The RMSE was estimated between all three data sets (manually tracked data vs. Schreiber's algorithm; manually tracked data vs. modified algorithm; Schreiber's algorithm vs. modified algorithm) as follows:

$$\boldsymbol{RMSE} = \sqrt{\frac{\sum_{i=0}^{n}(\boldsymbol{x_i} - \boldsymbol{y_i})^2}{\boldsymbol{n}}},$$

where x_i is the elongation of the tendon in millimeters at the frame number i of a certain data set. y_i, is the elongation of the tendon in millimeters, at the frame number i of the same video of another data set, and n is the total amount of frames. Additionally, the mean jumps for every video were estimated for the two algorithms. The mean jumps were estimated from the following formula:

$$\boldsymbol{Mj} = \sum_{i=0}^{n-1} \frac{\sqrt{(x_i - x_{i+1})^2}}{n}$$

Potential differences in the mean jumps between the initial and modified algorithm were examined by using a T-test for dependent samples. Furthermore, in order to determine the agreement between methods and examine any differences between the three tracking procedures with respect to the maximal GM_{tendon} elongation and tendon stiffness calculations, Bland-Altman plots (Bland and Altman 1999) and a one way analysis of variance (ANOVA), with the method as a factor, was used. Bonferoni's post-hoc comparison was performed when a significant main effect was detected. The level of significance was set at $\alpha = 0.05$. For both parameters, maximal GM_{tendon} elongation and tendon stiffness, the relationships between methods have been examined using a linear regression model. All results in the text and figures are presented as mean and standard error of mean. Furthermore, the range of the middle half of the scores (25th-75th percentile; interquartile range: IQR) was calculated for the analysed parameters and are provided in the text.

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Results

Examination of the excursion of the GM_{tendon} during the loading phase (see for example Fig. 3) revealed significantly lower mean jump values for the modified, in comparison to the automatic tracking algorithm (7.2±0.2 mm vs. 9.0±0.4mm per 10^3 frames; $P < 0.05$) with a time period of interest for all 132 videos of 6203 ± 116 ms (equal to 453 ± 9 frames). Furthermore, the absolute RMSE in tendon excursion during the loading phase between methods was lowest for the comparison between the manual and the modified algorithm (1.4 ± 0.1 mm) and highest between the manual and the Schreiber algorithm (2.0 ± 0.2 mm). For the comparison between the Schreiber algorithm and the modified algorithm, the RMSE in tendon excursion during loading was 1.8 ± 0.2 mm. The tracked GM_{tendon} elongation assessed with manual and the two automated tracking algorithms during MVIP is provided in Fig. 4. Concerning the maximal GM_{tendon} elongation during MVIP, there was no statistically significant method effect (manual tracking: 17.9 ± 0.3 mm; Schreiber algorithm: 17.0 ± 0.3 mm; modified algorithm: 16.9 ± 0.3 mm; Fig. 4). However, there was a tendency ($P = 0.054$) for lower tendon elongation values for the manual compared to both automated tracking methods. The Blant-Altman plots in Fig. 5C and 5D reveal that the mean differences or bias between measurements (manual method - automatic method) was 1.0 mm and 0.9 mm for the Schreiber and modified algorithm respectively and the 95% confidence intervals indicated that the maximum difference to manual tracking are higher for the Schreiber algorithm (7.4 mm) than for the modified algorithm (3.6 mm). Furthermore, the relationship between the three methods in maximal tendon elongation was significant ($P < 0.05$), with higher correlation values between the modified and manual algorithms ($R = 0.87$) than between the Schreiber and manual algorithms ($R = 0.56$; see Fig. 5A and 5B). There were no significant differences in tendon stiffness values between the manual (209 ± 4 N/mm) and modified algorithms (218 ± 5 N/mm) and the bland-Altman plot indicated that the mean difference or bias (manual method - automatic method) was -10 N/mm and that within the 95% confidence limits, the difference does not exceed 47 N/mm (Fig. 6D). In contrast to this, there were significantly ($P < 0.05$) higher tendon stiffness values generated by the Schreiber algorithm (229 ± 6 N/mm) in comparison to the manual method, and the Bland-Altman plot indicated that the mean difference or bias between measures (manual method - automatic method) is -21 N/mm and that within the 95% confidence limits, the difference can reach values up to 106 N/mm (Fig. 6C). As for the maximal GM_{tendon} during MVIP, the relationship between methods in tendon stiffness was significant ($P < 0.05$) with higher correlation values between the modified and manual algorithms ($R = 0.91$) than between the Schreiber and manual algorithms ($R = 0.52$; Fig. 6A and 6B). The IQR of the measurements was smaller for the modified algorithm (maximal tendon elongation: 3.7 mm; tendon stiffness: 64 N/mm) than for the Schreiber algorithm (4.6 mm; 76 N/mm) and, hence, closer to the values of the manual method (3.5 mm; 54 N/mm). In the same manner, for the bias between measures (difference between manual and automatic method) IQR was smaller for the modified (1.8 mm; 23 N/mm) than for the Schreiber algorithm (2.7 mm; 36 N/mm). Maximal calculated tendon force was on average 3657 ± 45 N and ranged between 1190 and 4430 N for the analysed 132 contraction trials.

Discussion

Although automatic tracking algorithms already exist (Lucas-Kanade 1981; Horn-Schunck 1981; Schreiber 2007), and are quite successful when tracking solid objects in good lighting conditions (Schreiber 2007; Baker and Matthews 2004), the accuracy of tracking algorithms for US videos examining human tendon length changes in vivo has not been thoroughly examined during voluntary contractions. Therefore, the main aim of the present work was the development and examination of a Lucas-Kanade optical flow based template tracking algorithm that would track GM_{tendon} elongation from US images during MVIP.

One of the difficulties that optical flow algorithms have to overcome is the fact that the appearance of objects on a video does not stay the same throughout a frame set. Speckle noise and violation of the constant intensity assumption add further difficulties to the estimation of optical flow in an US video. In the case of length changes of the GM_{tendon} during MVIP, it has to be kept in mind that the motion of the tendon of the US video is uniform and relatively slow. That led us to adding a jump penalty function to the algorithm, in order to eliminate any unwanted jumps in the tracking of the elongation of the GM_{tendon} Our results clearly demonstrate that this was achieved, since the current developed algorithm executed a mean of 72 µm jumps per frame when examining all of the 132 US videos, while Schreiber's (2007) initial algorithm produced significantly higher values with a mean of 90 µm jumps. Thus, our modified algorithm executed approximately 20% less jumps from frame to frame when examining GM_{tendon} elongation from US images during MVIP on a dynamometer, in comparison to the already existing Schreiber algorithm.

During an isometric ramp contraction, tendon elongation is uniform and slow and, hence, an algorithm that executes less jumps from frame to frame should be beneficial for following tendon excursion during loading more accurately. Accordingly, the RMSE of GM_{tendon} excursion during the loading phase shows that the current developed algorithm was closer to manual tracking (on average: 1.4 mm), than the RMSE from the Schreiber (2.0 mm). An analysis of the entire curve of the tendon excursing during MVIP on a dynamometer is particularly important for the examination of the force-length relationship of the tendon in vivo. Regarding this issue, it was found that the use of the Schreiber algorithm to track AT length changes during MIVIP resulted in a significant overestimation in tendon stiffness values when compared to manual tracking (229 ± 6 N/mm vs. 209 ± 4 N/mm), with a bias between measures of -21 N/mm. In contrast to this, tendon stiffness values generated by the modified tracking algorithm were not significantly different to manual tracking (218 ± 5 N/mm vs. 209 ± 4 N/mm). Moreover, there was a higher relationship in tendon stiffness between the modified algorithm and manual tracking (R = 0.91) than between the Schreiber algorithm and manual tracking (R = 0.52). Therefore, assuming that manual tracking is a valid method to examine tendon length changes during MIVP, the results of the current study suggest that the proposed algorithm (the first to directly compare tendon stiffness values generated with automatic tracking) can improve the assessment of tendon mechanical properties with dynamometric devices when using optical flow tracking algorithms.

When normalizing the RMSE by the total tendon excursion one might argue that the ~7% error found for the modified algorithm in the current study is similar to the results provided by Lee et al. (2008), who used optical flow to assess the displacement of the GM_{tendon} by US during a passive ankle joint motion. The authors reported errors of 6-8% in tendon displacement during passive ankle joint angular rotation using a similar manual tracking method as a reference. However, a passive ankle joint motion reduces movement dynamics of the triceps surae muscle-tendon unit, whereas in a voluntary maximal contraction condition, used in the current study, errors will likely be larger due to the GM_{tendon} being dynamically stretched during loading, leading to some deformation and making automatic tracking more difficult. In line with this suggestion, Pearson et al. (2013) recently reported that the automatic tendon tracking error found in their study was about 1.6 times higher during active compared to passive tests.

The tests reported here are the first to directly compare automated tracking with manually measured GM_{tendon} excursion during maximally loaded voluntary contractions in a large number of different US videos and using different tracking algorithms. To our knowledge, only one previous study discussed comparisons of highly loaded in vivo tendon excursions using an automated tracking method and manual measures (Pearson et al. 2013). The authors reported absolute errors in maximal GM_{tendon} elongation of up to 0.81 mm, which is lower to that seen on average here (about 0.9 mm). However, it has to be noted, that in the current study we examined 132 different US videos from 11 subjects. In contrast Pearson et al. (2013) only analyzed one subject, thereby neglecting potential differences in image quality across subjects that will affect the agreement or the ability of the algorithm to track regions effectively.

The Blant-Altman plots indicated that the mean differences in maximal GM_{tendon} elongation were only slightly lower for the current modified algorithm compared with the Schreiber algorithm and, therefore, it is reasonable to question whether the identified differences between our modified algorithm and Schreiber's algorithm is clinically or physiologically meaningful. However, the 95% confidence intervals indicated that the maximum differences to manual tracking are clearly higher for the Schreiber algorithm than for the modified algorithm (7.4 mm vs. 3.6 mm) with higher correlation values between the modified and manual algorithms (R = 0.87) than between the Schreiber and manual algorithms (R = 0.56). Moreover, our statistical test revealed higher tendon stiffness values for the Schreiber algorithm in comparison to the manual method with an average relative difference between methods of about 10%. In contrast, there was a higher agreement in tendon stiffness values between modified algorithm and manual tracking with an average relative difference between methods of about 5% and clearly lower difference in the 95% confidence (47 N/mm vs. 106 N/mm). Moreover, the modified algorithm, as opposed to the Schreiber algorithm, had lower measurement variability and reduced variability in the error compared to the manual method, as demonstrated by the lower IQR (up to 37% reduction) in maximal tendon elongation and tendon stiffness, indicating increased method robustness. We believe that such improvements in the accuracy and robustness of the method in AT length-tension property assessment are relevant and should not be neglected. In particular, when monitoring the time course of tendon mechanical changes resulting from injury, maturation, aging and altered mechanical loading, the identification of small changes in tendon mechanical properties is relevant for clinical and scientific settings.

There were several methodological drawbacks to this work which need to be noted. The 132 videos were captured as analog video and therefore, their qualities were influenced by converting them to different formats. This process severely impacted the quality of the tendon tracking. While this procedure is generally used for studying tendon biomechanical properties in vivo (Reeves et al. 2005; Arampatzis et al. 2007; Arya and Kulig 2010; Lee et al. 2008), due to raw data not usually being available from commercial US devices, future studies could try to use and analyse the radio frequency data. Another consideration is that we did not precisely control the rate of torque development and/or the time to reach peak joint moment during each ankle plantarflexion contraction. As a consequence, the number of US frames analysed for all examined 132 US videos ranged between 231 (minimum) and 700 (maximum) frames. However, as we used the same time region of interest for each video for all three methods, our main findings with respect to the comparison between tracking techniques will not be influenced. Fig. 3 shows that our method cannot eliminate noise-induced jumps completely, but it confines the jumps to a size controlled by the parameter d that roughly describes the typical step size tolerated by the algorithm. In our case, $d = 5$ corresponds to a jump size of 5 pixels, or a displacement of approximately 0.5mm. Both automatic methods fluctuate around the results obtained using the manual tracking method, but the fluctuations in our penalty based method are considerably smaller. Finally, one might argue that the lack of a test-retest reproducibility analysis of the modified tracking data weakens the current study. It is important to note that the data reported in this work were assessed on two separate sessions for each individual, with 6 US videos (6 contractions) from each session, originally performed in order to examine the test-retest reliability of the generated tendon length changes. However, when using such an analysis of tendon length changes during maximal voluntary muscle contraction, day-to-day physiological variation in muscle and tendon properties prevents an accurate assessment of the methods' reproducibility. For this reason we decided not to include the test-retest session analysis and pooled all data together. That being said, the examination of the test-retest reproducibility in tendon stiffness generated by our modified tracking algorithm showed no significant differences in tendon stiffness values between sessions (mean values session one: 220 ± 8 N/mm, range of data: 181 to 256 N/mm; session two: 215 ± 8 N/mm, range: 183 to 258 N/mm) and there was a significant correlation between the two sessions in tendon stiffness values with $R = 0.91$ ($P < 0.001$). Thus, we are confident that the modified tendon tracking algorithm is a valid measure of tendon length change and may be used to reliably examine Achilles tendon mechanical properties *in vivo*. Although not investigated, the current developed tracking algorithm is not restricted to a specific muscle-tendon unit and may, in the future, be applied to other tendons (e.g. quadriceps femoris tendon) in order to examine tendon and/or aponeurosis length changes during muscular contraction, as long as it is possible to identify a clear tissue landmark (e.g. myotendinous junction or the insertion of a fascicle into the aponeurosis).

In conclusion the results of this study suggest that the earlier Lucas-Kanade optical flow based template tracking algorithm proposed by Schreiber (2007) can be potentially used for non-subjective automatic estimation of the length changes of GM_{tendon} during MVIP in ultrasound images. However, adding a penalty function to the algorithm that eliminates unwanted jumps in the tracking of the elongation of the tendon can improve the estimation of GM_{tendon} elongation during MVIP on a dynamometer and hence, the assessment of in vivo tendon mechanical properties when compared with the established manual method. Further development and testing of image processing prior to application of the tracking algorithm is recommended to further improve the accuracy of the algorithm to estimate *in vivo* tendon displacement during maximal voluntary muscle contractions.

Conflict of interest statement

The authors have no conflicts of interest to report.

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Figure Captions List

Fig. 1: Schematic illustration of the experimental setup, including camera view (medial and lateral side) and the arrangement of the three strain gauge load cells fixed at predefined locations on the foot plate. The joint kinematic data in the sagittal plane and the force measurements were basically used to calculate the resultant ankle plantarflexion joint moments, and hence, tendon forces during contraction.

Fig. 2: Ultrasound images of the triceps surae muscle-tendon unit at rest (top) and at maximal gastrocnemius medialis tendon elongation (bottom) during the loading phase of a maximal ankle plantar flexion contraction. The red symbol represents the tracking node point.

Fig. 3: A typical trace of the gastrocnemius medialis tendon during a voluntary isometric ankle plantar flexion contraction on a dynamometer using the three methods (manual tracking, Schreiber's automatic tracking algorithm and the current modified tracking algorithm). The plot illsutartes that our developed algorithm cannot eliminate noise-induced jumps completely, but it confines the jumps to a size controlled by the parameter d that roughly describes the typical step size tolerated by the algorithm. In our case, $d = 5$ corresponds to a jump size of 5 pixels, or a displacement of approximately 0.5mm. Both automatic methods fluctuate around the results obtained using the manul tracking method, but the fluctuations in our penalty based method are considerably smaller. Please note that the subjects had to release their force after several seconds of holding the force at maximum and therefore, the tendon shortens again during the unloading phase (t > 6.5 sec).

Fig. 4: Mean (and standard error of mean; n=132) force-length relationship of the gastrocnemius medialis tendon from rest until maximal tendon force during voluntary isometric ankle plantar flexion contractions on a dynamometer estimated by the three

different tracking methods: manual tracking, the Schreiber's automatic tracking algorithm (Schreiber automatic) and the current modified Lucas-Kanade optical flow automatic tracking algorithm (Modified automatic) which was adapted to tendons' continuous and relatively slow movement characteristics by implementing a jump penalty function.

Fig. 5: Comparison of maximal gastrocnemius medialis tendon elongation during voluntary isometric ankle plantar flexion contractions on a dynamometer between tendon tracking methods. In the top two figures, the relationship between manual and the initial Schreiber's automatic tracking algorithm (A) and between manual tracking and the current modified automatic tracking algorithm (B) are presented. Bottom figures: In C (manual *vs.* Schreiber automatic tracking) and in D (manual *vs.* modified automatic tracking) the Bland-Altman plots showing the mean differences or bias between measures (manual method - automatic method) and 95% confidence limits. In total, 132 ultrasound videos were analyzed by the three methods.

Fig. 6: Comparisons of gastrocnemius medialis tendon stiffness values generated by the different tendon tracking methods. In the top two figures the relationship between manual and the initial Schreiber's automatic tracking algorithm (A) and between manual and the current modified automatic tracking algorithm (B) are presented. Bottom figures: In C (manual *vs.* Schreiber automatic tracking) and in D (manual *vs.* modified automatic tracking) the Bland-Altman plots showing the mean differences or bias between measures (manual method - automatic method) and 95% confidence limits. In total 132 ultrasound videos were analyzed by the three methods.