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Ultrasound-based assessment of gastrocnemius architecture during locomotion: analysis of fascicle tracking accuracy along the gait cycle.

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***Abstract*— Ultrasonography is a widely used technique for assessing muscle morphology by tracking fascicle length and pennation angle during contraction. In the last two decades, numerous automatic methods for fascicle tracking have been developed, but the majority of them were designed for static or well-controlled dynamic contractions, with limited applicability to unconstrained tasks, such as locomotion. The automatic fascicle tracking during these movements poses significant challenges, as out-of-plane movements of the fascicles can compromise their visibility in the bidimensional ultrasound image. In this study, we propose a semi-automatic tracking algorithm specifically designed to track fascicles during unconstrained cyclic movements. The approach integrates a frequency processing pipeline to enhance the visibility of the structures of interest and a supervision module specifically introduced to reduce tracking inaccuracies. We applied this method to ultrasound videos of the medial gastrocnemius in four subjects during walking. We then analyzed potential associations between gait phases and tracking errors relative to manual tracking. The results demonstrated the feasibility of the introduced tracking approach and a tendency of greater error occurrence during the stance-swing transition resulting from a diminished fascicle visibility.**

I. Introduction

Human gait is widely investigated due to its significant role in daily life. Impairments at any level of the neuromuscular pathway, from the command generation to the movement actuation, may have a strong impact on the locomotion, affecting the overall quality of life of individuals. Recently, in addition to the traditional assessments of kinematics and muscle activation, increasing attention has been given to the study of muscle architecture, as it offers important insights into muscle biomechanics which may be altered in various pathological conditions [1], [2]. Among the investigated muscles, the gastrocnemius medialis (GM) is particularly important for generating propulsive forces during locomotion and it is mainly active during the late stance phase of gait cycle with its major contribution during the push-off phase. Due to its accessibility and pennate structure, it has become the focus of numerous studies investigating muscle function during locomotion [1], [3].

Ultrasonography, due to its non-invasiveness, high degree of portability and usability, has been proposed to investigate muscle architecture during gait [4], [5], [6]. The tracking of pennate muscle architecture consists in the continuous estimation of fascicle length (FL) and pennation angle (PA) along the assessed movement. An automated tracking algorithm is warranted to offer a fast and accurate solution to study the time evolutions of FL and PA during gait cycles. Although manual tracking remains the gold standard for fascicle tracking, the manual identification in each frame of one or more fascicles is highly time-consuming, particularly when analyzing long tasks, such as gait analysis. Automatic alternatives to manual tracking have been mainly proposed for isometric or well controlled dynamic conditions such as isolated flexion-extension of a single joint [7], [8], [9], [10], [11]. However, unconstrained dynamic movements, such as walking, pose additional challenges for fascicle tracking due to the more complex morphological changes that muscles undergo during the contraction [12]. In particular, the three-dimensional architecture of the muscle cannot be fully captured using two-dimensional imaging. While this issue may occur for any type of contraction, it is particularly critical during unconstrained movements, as they are characterized by larger variability, greater range of motion and joint rotations across multiple anatomical planes. These factors increase the likelihood that the fascicle of interest moves out of the imaging plane, thereby compromising its visibility and consequently the tracking process. While these issues might be overcome by the expert eye of a human, the performance of an automatic tracking algorithm is often affected by the reduced fascicle visibility occurring in specific phases of the gait cycle.

Given the complexity and the cyclicity of the analyzed movement, the objective of this work is twofold: (i) to develop a new pipeline for GM fascicle tracking during walking, and (ii) to assess the effect of the gait phase on the fascicle tracking error.

II. Methods

A. Tracking algorithm

A new semi-automatic approach was adopted to analyze the ultrasonographic (US) data acquired during walking to extract the FL and PA (Figure 1). The fascicle tracking algorithm employs the Kanade-Lucas-Tomasi (KLT) algorithm to compute the optical flow to estimate the displacement of the feature points located on the regions of interest during dynamic and cyclic activities. It additionally integrates a frequency processing pipeline to enhance the visibility of the fascicles [13], and a supervision module that prompts operator intervention whenever possible tracking errors are identified.

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1) Preprocessing and structure initialization

Different image processing techniques were applied to improve overall image quality in terms of reduction of image noise (median filter), improvement of the contrast, enhancement of the brightness and improvement of edge definition (sharpening). Additionally, the Hough transform was computed for each frame to extract fascicle angular information only within the muscle belly region. The overall trend was then exploited in the supervision module (see 3) *Tracking and supervision controls*). Subsequently, the initialization of the structures of interest was performed on the first frame, starting with the automatic detection of the aponeuroses, followed by the manual annotation of a single fascicle by the user. The automatic detection of the aponeuroses was performed applying the Hough transform to a binarized and enhanced version of the image in which the aponeuroses were highlighted. Within each region of interest, feature points were detected exploiting the minimum eigenvalue algorithm and the scale-invariant feature transform algorithm implemented in two native Matlab functions (*detectMinEigenFeatures* and *detectSIFTFeatures*). The coordinates of these points were the starting point for the KLT algorithm.

2) Fascicle enhancement

This module was introduced to facilitate the tracking process by highlighting the visibility of the fascicles. During the tracking process for each frame the muscle belly was isolated exploiting the coordinates of the identified aponeuroses. Then, this region was processed in the frequency domain to extract the dominant orientations. The Differences of Gaussians technique [14] was preliminary applied to highlight areas corresponding to edges, then the fast Fourier transform was computed on a binarized version of the image. By thresholding the power spectrum and applying the inverse fast Fourier transform, the most prominent fascicles were effectively highlighted [13]. This frequency filter image was then multiplied to the original one so that only the fascicles resulted brighter, and then this result was superimposed to the total current frame.

3) Tracking and supervision controls

This block, exploiting the KLT algorithm, is responsible for the tracking of the feature points of each structure of interest through consecutive frames. A supervision module based on the Hough transform was introduced to overcome some common issues related to sequential tracking (i.e. drift) [9], [10], [11] giving the possibility to the user to manually correct the inclination and the position of the selected fascicle. Specifically, the whole Hough transform computed initially during preprocessing was used to identify possible inaccuracies and deviations from the physiological fascicle displacements. Indeed, even though the Hough signal is affected by a high degree of variability, and it is unsuitable for precise angular measurements, its filtered version can be employed as a rough reference of overall muscle architecture changes. We therefore used this information to monitor the quality of the tracking by introducing two supervision controls. It is worth noting that the control strategies described here assume the cyclicity of the movement (e.g. the gait) and are based on the detection of the peaks of the Hough transform, specifically:

i. The first one implemented a derivative analysis to detect opposite or alternating trends. In particular, the derivative of the Hough signal and the current automatic estimate of PA are compared, for each frame, within a predefined time window. If the number of discordant derivatives exceeds the 30% of the total number of samples of the time window considered, the user intervention is requested.
ii. The second one is specific for reducing inaccuracies during the shortening phase. In correspondence of each Hough angle peak the normalized difference between the two signals is computed and if it is higher than 10% the user intervention is requested.

The threshold values were selected after a preliminary analysis performed on the analyzed videos. However, every threshold can be changed according to the degree of accuracy desired considering that stricter thresholds would lead to higher manual interventions. Two additional controls, with adjustable thresholds based on the muscle being analyzed, were implemented, specifically based on the anatomy of the gastrocnemius muscle.

The first one verified that PA absolute values were within the anatomical range (i.e. not greater than 35° and not lower than 14° [15]). The second control examined the fascicle length trend between consecutive frames, aiming to prevent non physiological, abrupt changes. The threshold was set based on the average fascicle length range during walking [3], with a value not exceeding a quarter of this range.

B. Experimental validation

The study was conducted in accordance with the Declaration of Helsinki and the procedure approved by the Institutional Ethics Committee of Politecnico di Torino (reference number: 2772/2025). Informed consent was

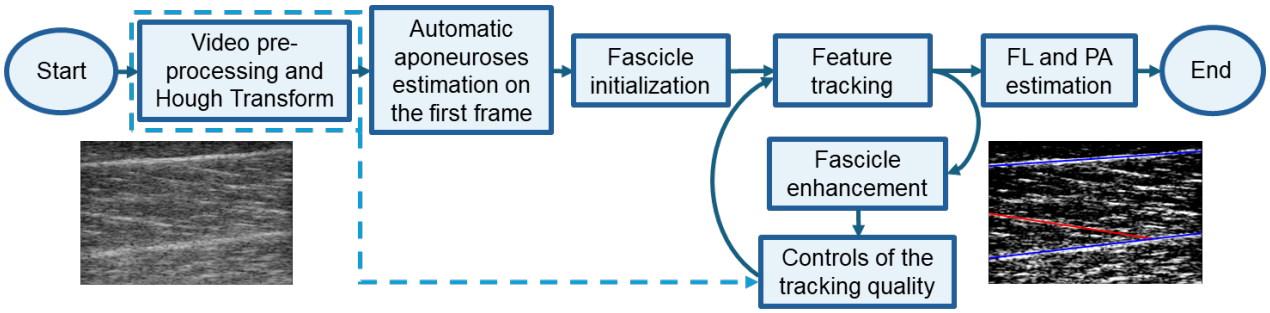


Figure 1. Block diagram of the proposed algorithm. During the pre-processing module, the Hough transform extracts the overall trend that is exploited in the supervision module within the tracking (dashed arrow). After the initialization steps (aponeuroses and fascicle), the KLT-based tracking integrates a fascicle enhancement module. The final output is the estimate of FL and PA in time.

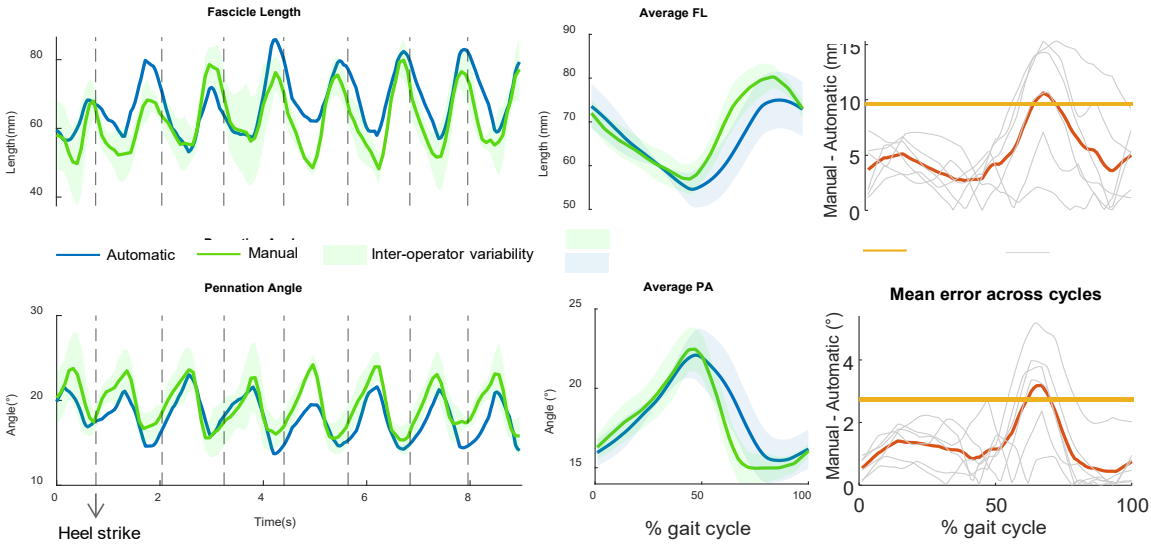


Figure 2. Left panel: Automatic (blue line) and manual (green line) estimates of a representative acquisition for FL and PA. The shaded green area indicates the inter-operator variability, and the dashed lines represents heel strike instants detected from the footswitch signal. Central panel: Average FL and PA waveforms across the gait cycle with their inter-cycle variability (shaded areas). Right panel: Representation of the difference between automatic and manual estimates (error) for each cycle (grey lines) and the overall mean (orange line). The yellow line indicates the error threshold determined for this subject.

obtained from all participants after receiving detailed explanation of the study procedures. US videos of the GM of four healthy subjects (1 male, 3 females; age 25 ± 1.4 yr) were acquired during walking on a treadmill at a constant speed of 4 km/h. A footswitch positioned under the heel was used to synchronize the videos with respect to the task cycles. Additionally, an external trigger was employed to synchronize the kinematic data to the US data acquired. The Verasonics Vantage™ research ultrasound system with a 128-element linear array probe (model L11-5v) was employed to acquire longitudinal scans of the GM at 500 fps. The probe was accurately fixed on the leg employing two elastic bands and a patch of bi-adhesive hydrogel between the probe and the skin.

C. *Data analysis*

B-mode image sequences were obtained beamforming the raw radiofrequency signals through the delay-and-sum (DAS) method and subsampling the original sequence to 125 fps. For each US video, three different expert operators performed the manual tracking and the mean of these three measurements was employed as a reference. The intraclass correlation coefficient (ICC) was computed to evaluate the inter-operator reliability based on a single rater, absolute-agreement, 2-way mixed-effects model [8]. The automatic estimates of FL and PA were compared to the manual references computing the root mean squared error (RMSE) for each video.

Manual and automatic measurements of both FL and PA were segmented in gait cycles using the heel strikes identified through the footswitch signal (dashed grey lines in left panel of Figure 2). The tracking error was defined as the average of the absolute difference between manual and automatic tracking for each cycle. For each subject, an error threshold was defined as the mean of the tracking errors across the cycles plus two times their standard deviation (yellow line in Figure 2 right panel). Each segmented cycle was then analyzed to identify data points exceeding this error threshold, resulting in the definition of a boolean vector. These boolean vectors were merged into a matrix (for all cycles and all subjects), and the sum of each column indicated the number of cycles that exceeded the error threshold for a given percentage of the gait cycle. These occurrences were normalized by the total number of gait cycles, and the results displayed in a histogram with a bin size of 10% of the gait cycle.

III. Results and Discussion

Figure 2 (left panel) depicts the automatic and manual estimates of a representative acquisition, with the shaded green area highlighting the inter-operator variability associated with manual tracking (ICC for FL: 0.81, ICC for PA: 0.81). The overall good agreement between the automatic and manual tracking across all the acquisitions is represented by an average RMSE of 5.85 ± 0.61 mm for FL and 1.51 ± 0.23 ° for PA.

The average FL and PA across cycles are represented in the central panel of Figure 2 and the cycles variability is indicated by the two shaded areas. For each cycle, the difference between automatic and manual curves is calculated and the result is shown in the right panel of Figure 2 while in orange the overall mean between cycles is represented. This representative example highlighted that the second half of the gait cycle was the most affected in terms of tracking accuracy for both PA and FL.

Figure 3 shows the normalized histograms of the count of cycles with error exceeding the subject-specific threshold. For the histogram associated to FL (upper panel), the highest levels are reached between the 60-90% of the gait cycle, whereas the histogram associated to PA presents a prominent peak in the bands of 60-70%. According to the literature [16] the 60% of the gait cycle delineates the beginning of the swing phase and the end of the stance phase. The activity of the GM is mainly concentrated in the first half of the gait cycle because of its strong contribution during the push-off phase [17]. Thus,

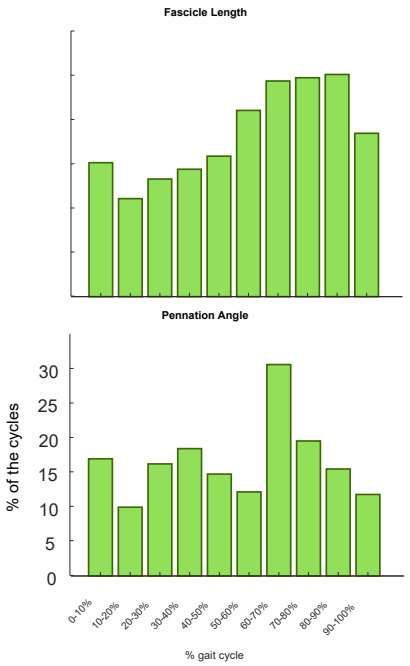


Figure 3. Histograms representing the percentage of the cycles with estimation errors exceeding the error threshold for both FL (upper panel) and PA (lower panel).

during the swing phase the GM is relaxing, and it undergoes a passive lengthening phase which is less controlled and can be influenced by additional rotations that can occur during transition between double support and single support. Considering that the probe was positioned on the muscle while the subject was still in an upright position, it is reasonable that in non-support conditions the fascicle of interest is likely to move out of the plane of the image thereby increasing the complexity of the tracking process in that transition phase between stance and swing.

IV. Conclusions

The algorithm presented in this study allowed to obtain estimation errors of PA and FL during locomotion comparable to those observed in more controlled experimental conditions [8], [11]. It was possible to show that the estimation error of architectural variables depends on the gait phase, with larger errors occurring during the transition between stance and swing. However, due to the limited number of subjects involved, this evidence should be confirmed on a larger experimental sample due to the high intersubject variability both related to anatomical differences and different gait patterns. This preliminary result suggests that care must be taken when assessing the fascicle tracking output throughout the entire cycle of unconstrained tasks, such as gait. However, when compared to manual tracking, the proposed approach offers a good tradeoff between analysis time and accuracy, opening up new possibilities for investigating fascicle movements during the gait cycle and their alterations.

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