

Insights gained into the interpretation of surface electromyograms from the gastrocnemius muscles: A simulation study

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1 **Title:** Insights gained into the interpretation of surface electromyograms from the gastrocnemius  
2 muscles: a simulation study.

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17

1 **ABSTRACT**

2 Interpretation of surface electromyograms (EMG) is usually based on the assumption that the  
3 surface representation of action potentials does not change during their propagation. This  
4 assumption does not hold for muscles whose fibers are oblique to the skin. Consequently, the  
5 interpretation of surface EMGs recorded from pinnate muscles unlikely prompts from current  
6 knowledge. Here we present a complete analytical model that supports the interpretation of  
7 experimental EMGs detected from muscles with oblique architecture. EMGs were recorded from  
8 the medial gastrocnemius muscle during voluntary and electrically elicited contractions.  
9 Preliminary indications obtained from simulated and experimental signals concern the spatial  
10 localization of surface potentials and the myoelectric fatigue. Specifically, the spatial distribution of  
11 surface EMGs was localized about the fibers superficial extremity. Strikingly, this localization  
12 increased with the pinnation angle, both for the simulated EMGs and the recorded M-waves.  
13 Moreover, the average rectified value (ARV) and the mean frequency (MNF) of interference EMGs  
14 increased and decreased with fatigue, respectively. Furthermore, the degree of variation in ARV and  
15 MNF did not depend on the pinnation angle simulated. Similar variations were observed for the  
16 experimental EMGs, although being less evident for a higher fiber inclination. These results are  
17 discussed on a physiological context, highlighting the relevance of the model proposed here for the  
18 interpretation of gastrocnemius EMGs and for conceiving future experiments on muscles with  
19 pinnate geometry.

20

## 1 **1. INTRODUCTION**

2 The modeling of surface electromyograms (EMGs) has been sought for the interpretation of  
3 experimental data (Dimitrova and Dimitrov 2003; Merletti et al., 1999b; Roeleveld et al., 1997), for  
4 the development of algorithms aimed at information extraction (Duchene and Hogrel, 2000; Mesin  
5 et al., 2009), and for didactic purposes (Merletti et al., 1999a). Available models for the generation  
6 of surface EMGs rely both on numerical (Lowery et al., 2004; Mesin et al., 2006) and analytical  
7 (Block et al., 2002; Farina et al., 2004b; Gootzen et al., 1991; Mesin, 2006) approaches.

8  
9 The assumption that the volume conductor is invariant in the direction of propagation of  
10 intracellular action potentials allowed for the development of fast analytical models for the  
11 simulation of surface EMGs (Farina and Merletti, 2001; Farina et al., 2004a). If a volume conductor  
12 is space invariant, the surface representation of a motor unit action potential does not change with  
13 its propagation. Such space invariance is frequently assumed when simulating, processing and  
14 interpreting experimental EMGs (Lindstrom and Magnusson, 1977; Reucher et al., 1987).  
15 Therefore, much of the insights gained into the interpretations of surface EMGs from the use of  
16 mathematical models are valid, exclusively, for muscles whose geometry fits in the assumption of  
17 space invariance.

18  
19 Some of the muscles investigated by surface EMG cannot be approximated assuming the muscle  
20 fibers to be parallel to the skin. The fibers of the gastrocnemius muscle, for example, are inclined  
21 with respect to the skin surface and extend from the deep to the superficial aponeurosis (Kawakami  
22 et al, 1998; Narici et al., 1996). Surface electrodes positioned on the calf are, thus, located above the  
23 superficial aponeurosis, where muscle fibers attach. Considering that action potentials propagate  
24 along the muscle fibers, their propagation along the oblique gastrocnemius fibers contributes to the  
25 surface EMGs with a component toward muscle extremities and another toward the skin/tibia.  
26 Therefore, the area on the skin surface upon which the action potential of a single muscle fiber

1 distributes likely depends on how inclined the muscle fibers are (i.e. its pinnation angle) (Vieira et  
2 al., 2011). Currently existing models do not provide indications of how the surface EMG relates to  
3 the pinnation angle. Which physiologically relevant information might be extracted from surface  
4 EMGs in the pinnate gastrocnemius muscle is unknown.

5  
6 In this study we simulate a pinnate muscle, with fibers inclined in the depth direction, to interpret  
7 surface EMGs detected from the gastrocnemius muscles. A complete mathematical model, which  
8 includes muscle fibers with finite length and three layers of tissues, is presented here for the  
9 generation of single fiber action potentials (SFAP). In particular, we simulate the distribution of  
10 motor unit action potentials (MUAPs) on the skin and the interference EMG for different degrees of  
11 inclination of muscle fibers. The implications for the interpretation of how action potentials  
12 distribute on the skin surface and for the estimation of muscle fatigue are addressed as well.  
13 Ultrasound images and experimental EMGs are recorded from the human medial gastrocnemius  
14 (MG) muscle to investigate how much theoretical and empirical data correspond.

15

## 16 **2. METHODS**

### 17 **2.1 Mathematical model**

18 The simulation model was developed by extending our previous work (Mesin and Farina, 2004),  
19 which considered the impulse response of a two layer volume conductor. Three layers were  
20 considered here (skin, fat and muscle; figure 1). Moreover a complete model (including finite-  
21 length fibers) was implemented to generate single fiber action potentials (SFAP).

22

#### **Figure 1**

23 Libraries of MUAPs were generated from the simulated SFAPs. These libraries were used with a  
24 model of spatial and temporal recruitment of motor units (MUs) to simulate interference EMGs  
25 during fatiguing contractions.

26

1 Full details on the model and the simulated EMGs are described in the Appendix.

2

## 3 **2.2 Experimental signals**

4 Single-differential EMGs were recorded from the medial gastrocnemius (MG) muscle to test for the  
5 correctness of the information gained from simulated signals. In particular, we investigated how the  
6 amplitude of surface potentials varies with the pinnation angle. This is of remarkable interest since  
7 this variation likely provides information of how localized the gastrocnemius activity might be.  
8 Moreover, we were interested in understanding whether experimental and simulated EMGs in  
9 pinnate muscles are comparable during fatiguing contractions.

10

11 Three male subjects (age: 33, 29, 27years; body mass: 78, 80, 75kg; height: 182, 178, 180cm)  
12 participated in two protocols designed to compare experimental and simulated signals.

13

14 Protocol 1: Sixteen surface electrodes (10 mm IED) were used to record EMGs during electrical  
15 stimulation. Two ankle angles were considered; foot in neutral position ( $\sim 20^\circ$  pinnation angle) and  
16 plantar flexed ( $\sim 35^\circ$  pinnation angle). With an adhesive pre-gelled electrode (cathode; figure 6a)  
17 placed carefully on the leg, bipolar current pulses were delivered at 2 pps and for 20s to the tibial  
18 posterior nerve. The anode electrode ( $80 \times 50$  mm; soaked cloth) was positioned immediately above  
19 the patella with elastic Velcro straps. Stimulation amplitude was as minimal as possible to allow for  
20 the detection of the firstly observable M-wave. Low stimulation amplitude was chosen to recruit the  
21 least number of MUs and, thus, to better isolate the effect of ankle angle on the distribution of M-  
22 waves amplitude on the skin. Averaged M-waves were obtained after triggering the 15 single-  
23 differential EMGs.

24

25 Protocol 2: EMGs were recorded with an array of eight electrodes (5mm interelectrode distance –  
26 IED) when the subjects exerted isometric plantar flexion at 60% MVC. The contraction lasted for

1 30s to ensure the occurrence of myoelectric manifestation of fatigue. Fatigue plots were created  
2 from the average rectified value (ARV) and the mean frequency (MNF) calculated on 500ms  
3 epochs (i.e. both descriptors are plotted with respect to their initial values). ARV and MNF indices  
4 were calculated as indicated in Merletti et al. 1990. This protocol was applied once with the foot  
5 dorsal flexed (pinnation angle  $\sim 10^\circ$ ) and once in neutral position (pinnation angle  $\sim 20^\circ$ ), with 5 min  
6 interval between trials. Plantar flexion torque was measured and displayed to the subjects. Subjects  
7 were in prone position and their feet were firmly secured to a footplate, with the lateral malleolus  
8 being coaxial to the centre of rotation of the torque meter. MVC values were determined for each  
9 pinnation angle as the maximum torque measured across three attempts separated by 5 minutes.

10

11 EMGs were amplified (gain ranged from 1k to 5k; 10–500 Hz EMG-USB amplifier, LISiN and  
12 OTBioelettronica, Turin), and sampled at 2048 Hz with a 12bit A/D converter ( $\pm 2.5V$  dynamic  
13 range). Ultrasound images were taken with a linear probe (3.86 cm long; Fukuda Denshi, UF 4000,  
14 7.5 MHz) and pinnation angles were estimated with the precision of one degree. A custom-made  
15 neuromuscular stimulator (LISiN, Turin), equipped with a hybrid output stage, was used in the first  
16 protocol. In the second protocol, load cells output was amplified (150Nm full-scale amplifier;  
17 MISOI, OTBioelettronica, Turin) and then converted to values of ankle torque. After cleansing of  
18 the skin with abrasive paste and water, ultrasound scanning was used to place electrodes on the MG  
19 muscle. Specifically, electrodes were positioned above the sheath of aponeurotic tissue and parallel  
20 to the surface projection of the pinnate MG fascicles. Care was taken to ensure that the array and  
21 the ultrasound probe had similar orientation, which provided an ultrasound image with as many  
22 fascicles as possible.

23

## 24 **3. RESULTS**

### 25 **3.1 Preliminary Simulations**

1 Some preliminary simulations are shown in figures 2 and 3, in order to compare the surface  
2 potentials produced by fibers with different arrangements (parallel or slightly inclined with respect  
3 to the skin). In particular, the potential distribution over the skin surface is considered for fixed time  
4 samples in figure 2, whereas the signals detected as functions of time from arrays of electrodes  
5 sampling the potential in specific points aligned to the fibers are shown in figure 3.

6  
7 Simulations showed that the amplitude and the shape of surface potentials change with the obliquity  
8 of muscle fibers (figure 2). When simulating fibers parallel to the skin, the surface distribution of  
9 action potentials was symmetric with respect to the innervation zone (IZ). Conversely, by  
10 increasing the fiber inclination, the surface potential became more asymmetric, as the contribution  
11 of the deeper source was attenuated and more diffused than that of the most superficial source. The  
12 root mean square (RMS) difference between the surface distribution of potentials simulated for  $10^\circ$   
13 and those computed for the other pinnation angles ( $0^\circ$ ,  $5^\circ$ ,  $15^\circ$ , and  $20^\circ$ ) is shown as a function of  
14 the source position (figure 2b). As expected, greater RMS differences were obtained for higher  
15 variations between angles. The greatest difference occurred when the source was close to the  
16 extinction region.

17  
18 Regardless of whether simulating monopolar or single-differential signals, variations in the  
19 amplitude of surface potentials across electrodes depended on the pinnation angle. By simulating an  
20 electrode array placed parallel to the x-z projection of a superficial muscle fiber with  $0^\circ$  pinnation  
21 angle, both monopolar and differential potentials appeared with equal amplitudes on either sides  
22 from the IZ (left panel in figure 3a). For deep fibers, only the standing waves produced by the  
23 generation and extinction of simulated potentials are seen in the EMGs, respectively (right panel in  
24 figure 3a). By inclining muscle fibers, the amplitude distribution of surface potentials concentrated  
25 progressively more toward the superficial tendon (left panels in figure 3b,c). Interestingly, pinnate  
26 fibers simulated 10cm away from the electrodes contributed to the surface EMGs with potentials of

1 markedly smaller amplitude (3-5 times smaller; right panels in figure 3b,c). Therefore, surface  
2 electrodes on pinnate muscles sample only from sources located nearby and nowhere else.

3  
4 The inclination of muscle fibers affects also the shape and the propagation of surface potentials.  
5 Close inspection of figure 3 reveals that the surface potential from fibers parallel to the skin has the  
6 same duration, wherever it is sampled on the skin. Propagation of these potentials is also evident in  
7 the figure. Potentials from pinnate fibers undergo large shape variations when recorded from  
8 different channels, so that conduction velocity (CV) cannot be estimated properly (Farina and  
9 Merletti, 2004). Specifically, they have shorter duration when detected closer to the superficial  
10 tendon. Moreover, the delay between successive peaks or valleys of potentials simulated for  
11 adjacent channels is not the same for pinnate fibers. Then, surface EMGs detected from muscles  
12 with fibers not parallel to the skin unlikely show the propagation of action potentials along muscle  
13 fibers.

### 14 **Figures 2 and 3**

15

### 16 **3.2 Amplitude distribution of EMGs**

17 In figure 4, simulated single fiber potentials are investigated to quantify how much the spatial  
18 distribution of surface EMGs changes as a function of the pinnation angle. Surface potentials were  
19 compared for volume conductors with various pinnation angles, from 0° to 45°, and for two  
20 thicknesses of the fat layer. Contour plots of simulated surface potentials are shown at a given time  
21 (figure 4b). To quantify the diffusion of the surface potential, the median and the first and third  
22 quartiles were investigated along orthogonal sections of contour plots (figure 4c). The potential  
23 generated using a model of parallel fibers was more diffused (interquartile interval: ~40mm) over  
24 the skin with respect to that generated with a pinnate geometry (interquartile interval: less than  
25 25mm for pinnation angles higher than 10°; figure 4).

26

1 As for the simulated SFAPs, the amplitude distribution of electrically elicited EMGs depended on  
2 the degree of inclination of MG fibers. When MG fascicles were  $\sim 20^\circ$  oblique, the firstly emerging  
3 M-waves appeared on the most proximal muscle portion, from the channel 1 to 5 (figure 5b). The  
4 ARV amplitude of M-waves distributed evenly across these channels (interquartile interval:  
5 53.7mm). Strikingly, for the same stimulation amplitude and higher pinnation angle ( $\sim 35^\circ$ ), the  
6 distribution of ARV amplitude changed chiefly across channels (interquartile interval: 32.2mm),  
7 with the most proximal channels detecting the largest M-waves (figure 5c). Additionally, M-waves  
8 recorded from the pinnate gastrocnemius muscle showed neither a delay nor a phase opposition  
9 between consecutive channels.

#### 10 **Figure 4 and 5**

### 11 **3.3. Simulated and experimental manifestation of fatigue in surface EMGs**

12 Simulations indicate that amplitude and spectral descriptors might be used to study the myoelectric  
13 manifestations of fatigue in the pinnate gastrocnemius muscle. Single-differential EMGs, simulated  
14 and recorded with seven channels aligned to the longitudinal projection of muscle fibers, are shown  
15 for two pinnation angles ( $10^\circ$  and  $20^\circ$ ; figure 6). Both simulated (session 2.4) and experimental  
16 signals showed myoelectric manifestation of fatigue, with ARV and MNF increasing and  
17 decreasing with time, respectively (fatigue plots; figure 6). Interestingly, changes in amplitude and  
18 frequency were more variable for the experimental EMGs; and this high variability increases with  
19 the pinnation angle (Table 1).

#### 20 **Figure 6, Table 1**

## 21 **4. DISCUSSION**

22 A model for the simulation and interpretation of surface EMGs in pinnate muscles is proposed here.  
23 Simulations indicate that the obliquity of muscle fibers has a marked effect on the surface EMG  
24 (figure 2). The higher the fibers pinnation the more localized was the surface distribution of action  
25 potentials, with electrodes closer to the fibers end detecting remarkably higher potentials. This  
26 localized distribution of surface potentials is more evident for single-differential than for monopolar

1 signals, likely due to the higher selectivity of differential derivations. Experimental EMGs from the  
2 MG substantiated the predictions posed by our simulations.

3  
4 While there are architectural differences between skeletal muscles, it seems worthy to ask whether  
5 the surface EMGs recorded from pinnate muscles are as informative as those recorded from  
6 fusiform muscles. Understanding how the amplitude distribution of surface potentials changes with  
7 the pinnation angle and the possibility of using surface EMGs to study muscle fatigue are of  
8 particular interest.

#### 9 10 **4.1 Localization of surface EMGs depends on the pinnation angle of the gastrocnemius muscle**

11 Our simulations revealed that surface electrodes on muscles with fibers not parallel to the skin  
12 sample from nearby sources and from nowhere else. This key result is substantiated from our  
13 observations that: i) surface potentials were localized over the superficial tendon of pinnate fibers,  
14 with the main contribution being due to the extinction phase of intracellular action potentials (figure  
15 3); ii) although the representation of surface potentials was 50% less localized when trebling the fat  
16 thickness, it was still considerably more concentrated on the superficial tendon than the surface  
17 representation of potentials in parallel fibers (figure 4); iii) two pinnate fibers with distal tendons  
18 separated by 10cm produced surface potentials with separated amplitude distributions.  
19 Consequently, the pinnation brings a wider cross section of the muscle into the view of surface  
20 electrodes than that available for parallel-fibered muscles. It is worth to mention that the localized  
21 distribution shown in figures 3 and 4 refers to the amplitude of SFAPs. As the amplitude of MUAPs  
22 corresponds to the algebraic summation of several SFAPs, the localized representation of individual  
23 MUAPs in the surface EMGs depends on whether MUs have small territories (Vieira et al., 2011),  
24 Here, we are not interested on the size of MUs territories but on how much the amplitude  
25 distribution of SFAPs changes with the pinnation angle.

26

1 Electrical stimulation of the tibial nerve revealed a somewhat similar localization of surface M-  
2 waves detected from the MG muscle (figure 5), with respect to that observed for simulated  
3 potentials. The smallest stimulation amplitude leading to the first observable M-waves was chosen  
4 to ensure that only a few MUs were stimulated. By keeping constant the stimulation amplitude, the  
5 effect of pinnation angle on the localization of M-waves was isolated. M-waves with similar  
6 amplitude were observed for the four most proximal channels when stimulation pulses were  
7 delivered with the foot in neutral position (figure 5b,  $\theta = 20^\circ$ ). With the foot plantar flexed (figure  
8 5c,  $\theta = 35^\circ$ ), the amplitude of M-waves distributed unevenly across the four most proximal  
9 channels. Larger M-waves appeared for the more proximal channels. While the variation in ankle  
10 joint angle could have induced variation in the stimulation site, as the nerve moves beneath the  
11 stimulation electrode, the similitude of EMGs likely indicates that the same population of MUs was  
12 stimulated in both foot conditions.

13

14 Surface EMGs convey unique information regarding the activation of the pinnate gastrocnemius  
15 muscle. In muscles whose fibers are parallel to the skin, surface electrodes detect the propagation of  
16 MUAPs (Farina et al., 2002; Merletti et al., 2003). In the gastrocnemius muscle, the amplitude of  
17 surface EMGs varies with the number of active fibers beneath the recording electrodes. Very  
18 recently, for example, we used the model presented here to validate our estimations of the  
19 longitudinal size of MUs territory in the human MG muscle (Vieira et al., 2011). Specifically, we  
20 observed that with respect to the MG length, the fibers of individual MUs extended for only a short  
21 distance (less than 4cm). Then, activation of individual MUs leads to regional activation of the MG  
22 muscle. Indeed, the extensive evidence positing localized activation of the calf muscles in humans  
23 and cats is not surprising (Eng and Hoffer, 1997; English and Weeks, 1989; McLean and Goudy,  
24 2004; Staudenmann et al., 2009; Vieira et al., 2010a,b; Wolf et al., 1998). If the nervous system  
25 takes advantage of muscle architecture to shape the recruitment of MUs (Kennedy and Cresswell,

1 2001; Vieira et al., 2011), the high-density surface electromyography (Merletti et al., 2010) could  
2 provide a mean to study the regional organization of activity in the pinnate gastrocnemius muscle.

3

#### 4 **4.2 Myoelectric manifestation of fatigue in pinnate muscles**

5 Testing for the possibility of studying fatigue manifestation in skeletal muscles from the surface  
6 EMG is not simple. Several factors affect the muscles undergoing fatigue: 1) different strategies of  
7 MU recruitment and firing rate; 2) variations in load sharing between synergistic muscles,  
8 especially for those spanning the same joint as soleus and gastrocnemius (McLean and Goudy,  
9 2004); 3) changes in shape of intracellular action potentials (Arabadzhev et al., 2005); 4)  
10 synchronization of MUs (Mesin et al., 2009); 5) variable decrease of CV for muscle fibers of  
11 different physiological types (Rainoldi et al., 2008). To keep simulations simple, we considered the  
12 same percentage of CV changes for all MUs. Additionally, the same spatial and temporal  
13 recruitment of MUs was simulated for two different pinnation angles, so as to isolate the effect of  
14 fibers inclination on surface EMGs.

15

16 Even though it was not possible to observe the changes in CV directly, it was possible to observe  
17 myoelectric manifestations of gastrocnemius fatigue in the amplitude and spectral EMG descriptors.  
18 Because of the muscle oblique architecture, and considering the scattering of IZs, the extinction of  
19 action potentials at the superficial aponeurosis contributed chiefly to the surface EMGs simulated  
20 (figure 3). As a consequence, electrodes located over the aponeurotic layer do not detect the same  
21 potential propagating toward either the superficial or deep aponeurosis. Instead, they record  
22 standing waves likely resulting from the end-of-fiber effect (Stegeman et al., 1997). This  
23 interpretation is supported by spike-triggered EMGs, which did not show physiological delays  
24 between surface potentials detected by consecutive electrodes located upon the superficial  
25 aponeurosis (Vieira et al., 2011). Thus, estimation of CV, which showed remarkable properties in  
26 the investigation of myoelectric fatigue in parallel-fibered muscles (Mesin et al., 2009), cannot be

1 obtained from the gastrocnemius muscle, at least not upon its superficial aponeurosis. Nevertheless,  
2 simulations showed that ARV and MNF of surface EMGs are both affected by variations in CV.

3  
4 Although at different extents, the experimental and simulated manifestations of fatigue were  
5 observed in the pinnate gastrocnemius muscle. Simulations did not show any reliance of the  
6 variations in ARV and MNF on the pinnation angle. Nevertheless, the variability of ARV and MNF  
7 were different for experimental data recorded for two pinnation angles (Table 1). This suggests that  
8 some factors, which were kept constant in the simulations, were involved in the contraction of the  
9 MG muscle at different pinnation angles. It has been shown, for example, that variations in activity  
10 within and between calf muscles occur when subjects are asked to sustain a low and isometric  
11 torque of plantar flexion (McLean and Goudy, 2004; Tamaki et al., 1998). Variable activation  
12 within the same gastrocnemius muscle was observed even for different directions of ankle force  
13 (Staudenmann et al., 2009) and in quiet standing (Vieira et al., 2010a,b). Considering the more  
14 localized distribution of surface potentials for the more oblique geometry (figures 4), and the small  
15 territories of gastrocnemius MUs (Vieira et al., 2011), surface electrodes seem to provide very  
16 selective recordings for high pinnation angles. Therefore, variations in recruitment and firing rate of  
17 MUs during a fatiguing contraction would affect more severely the surface EMGs detected from  
18 more oblique MG fibers.

19

### 20 **4.3 Future perspectives**

21 The model presented here opens new perspectives for investigating the calf muscles activation,  
22 either by supporting the interpretation of experimental data or by providing theoretical grounds  
23 upon which future studies will be designed. The surface distribution of simulated action potentials  
24 (figures 3 and 4), for example, supports our prediction that postural activation of the MG muscle is  
25 organized regionally for the control of quiet standing posture (Vieira et al., 2010a). Similarly, our

1 simulation results indicate that amplitude and spectral EMG descriptors could be potentially useful  
2 to study fatigue in the gastrocnemius muscle.

3

## 4 **5. CONCLUSIONS**

5 A new model for the simulation of surface EMGs in pinnate muscles was developed and applied to  
6 interpret experimental data obtained from the gastrocnemius muscles. The most striking result was  
7 the localized representation of surface potentials. Because of the gastrocnemius pinnate  
8 architecture, simulated EMGs and experimental M-waves were both detected only by few  
9 consecutive electrodes. Higher pinnation angles led to more localized potentials. The regional  
10 organization of gastrocnemius activity in different motor tasks could, then, be investigated with  
11 surface electromyography. The potentiality of the model was also shown for the investigation of  
12 myoelectric fatigue with amplitude and spectral indexes. Both descriptors varied during fatigue,  
13 either for experimental or simulated EMGs. The greater variability in the amplitude and frequency  
14 of experimental EMGs, observed for higher pinnation angles, was likely due to different  
15 recruitment strategies.

16

## 17 **Conflict of Interest Statement**

18 None of the authors has any conflict of interest concerning the publications of this work.

19

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24

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1 **Table 1:** Variability of the amplitude (ARV) and frequency (MNF) descriptors of experimental and  
2 simulated surface EMGs, calculated as the standard deviation of residuals resulting from the fitting of  
3 a first order polynomial to the observed data (dashed lines in figure 4).

	Experimental EMGs		Simulated EMGs	
Pinnation angle (deg)	ARV ( $\mu\text{V}$ )	MNF (Hz)	ARV (a.u.)	MNF (Hz)
10	67	4.16	4.21	3.61
20	108	4.68	4.20	3.62

4

5

1 **FIGURE CAPTIONS**

2 **Figure 1** a) Schematic representation of the volume conductor model, including the definition of  
3 the coordinate system and the geometry of the simulated muscle fibers. The muscle layer is  
4 considered as homogeneous and anisotropic, with fibers inclined with respect to the skin surface.  
5 Fat and skin layers are homogeneous and isotropic. b) Ultrasound image of the gastrocnemius,  
6 showing the pinnation of muscle fibers. c) Longitudinal section of the simulated volume conductor,  
7 indicating the notation used for determining the analytical solution. d) Sampling of the  
8 phenomenological model of the transmembrane current proposed in Rosenfalck (1969), using 10  
9 impulse sources.

10 **Figure 2** a) Surface potential generated in muscle fibers with three different pinnation angles ( $0^\circ$ ,  
11  $5^\circ$ , and  $10^\circ$ ). The signals were simulated using the model shown in Figure 1a,c. b) Root mean  
12 square (RMS) difference between the EMG map generated for the pinnation angle of  $10^\circ$  and the  
13 maps computed for pinnation angles of  $0^\circ$ ,  $5^\circ$ ,  $15^\circ$ , and  $20^\circ$ . Two different thicknesses of the fat  
14 layer were simulated. RMS values are expressed in percentage and as a function of the position of  
15 the source (measured as the distance of each of the two transmembrane current sources from the  
16 innervation zone).

17 **Figure 3** Simulation of surface EMG signals detected with an electrode array located over the  
18 longitudinal projection of a single muscle fiber on the skin surface. Monopolar and single-  
19 differential signals are shown for different fibers, corresponding to different pinnation angles or to  
20 different positions.

21 **Figure 4** Simulated (a; see Appendix) and experimental (b; see section 2.2) EMGs are shown for  
22 two pinnation angles ( $10^\circ$  and  $20^\circ$ ) during an isometric fatiguing contraction at 60% of maximal  
23 voluntary contraction (MVC). Interference EMGs were simulated with the muscle fiber conduction  
24 velocity - CV - decreasing by 1%/s of the initial value. The volume conductors simulated and  
25 ultrasound images of the MG muscle are shown on top (arrows indicate detection points). The same  
26 firing pattern and population of 340 motor units were simulated for two pinnation angles ( $10^\circ$  and

1 20°). A short time epoch of raw signals is depicted in the middle panel. Fatigue plots are shown in  
2 the bottom, including averaged rectified values (ARV) and mean frequency (MNF), estimated for  
3 epochs of 500 ms duration (n = 60 epochs) and for each pinnation angle. Values were averaged  
4 across channels (n = 7 channels). Dashed lines indicate the best linear fitting to ARV and MNF  
5 traces, calculated with the least square method.

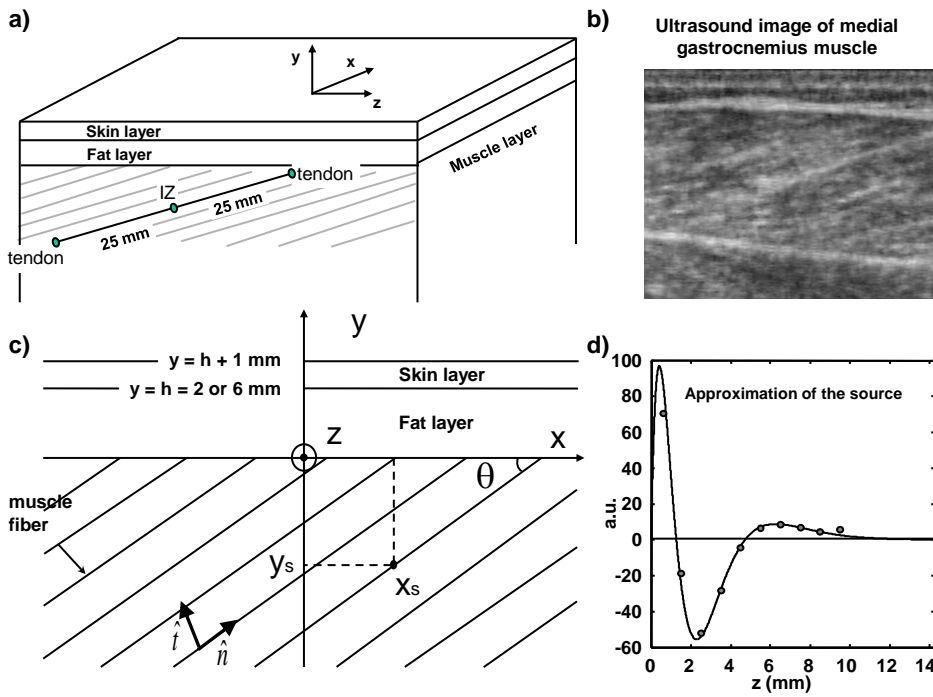
6 **Figure 5** Spatial distributions of surface potentials as a function of the pinnation angle. a)  
7 Longitudinal view of the volume conductors considered. When the pinnation angle is higher than  
8 zero, one of the tendons is located at the muscle/fat interface, representing the superficial  
9 aponeurosis. The case in which fibers are parallel to the skin surface is also considered, for two  
10 muscle depths. b) Contour plots of the rectified value, averaged over time (ARV), of the simulated  
11 surface potential, indicating the median and the first and third quartiles for orthogonal sections  
12 crossing the location of maximal amplitude. Note the asymmetry of contours for pinnation angles  
13 greater than zero; ARV amplitude concentrates close to the fiber end (the superficial aponeurosis).  
14 c) Median, first and third quartiles of ARV in the direction longitudinal (x) and transversal (z) to the  
15 fibers are shown for different pinnation angles and for two fat layers.

16 **Figure 6** Spatial distribution of single-differential M-waves detected from the medial  
17 gastrocnemius (MG) muscle at two pinnation angles. a) Array of 16 surface electrodes, positioned  
18 on the MG muscle, and the stimulation electrode, located above the tibial nerve branch supplying  
19 the same muscle. Note that the most proximal EMG electrodes were nearer to the stimulation  
20 electrode. b) Raw triggered (black traces) and averaged (gray traces) M-waves detected with the  
21 foot positioned so as to result in a pinnation angle of about 20°. The ultrasound image obtained with  
22 the foot in this same position is shown on the top. c) the same as in b) for ~35° pinnation angle.  
23 Note that the stimulation artefact was most evident for the most proximal channels, which were the  
24 closest to the cathode electrode.

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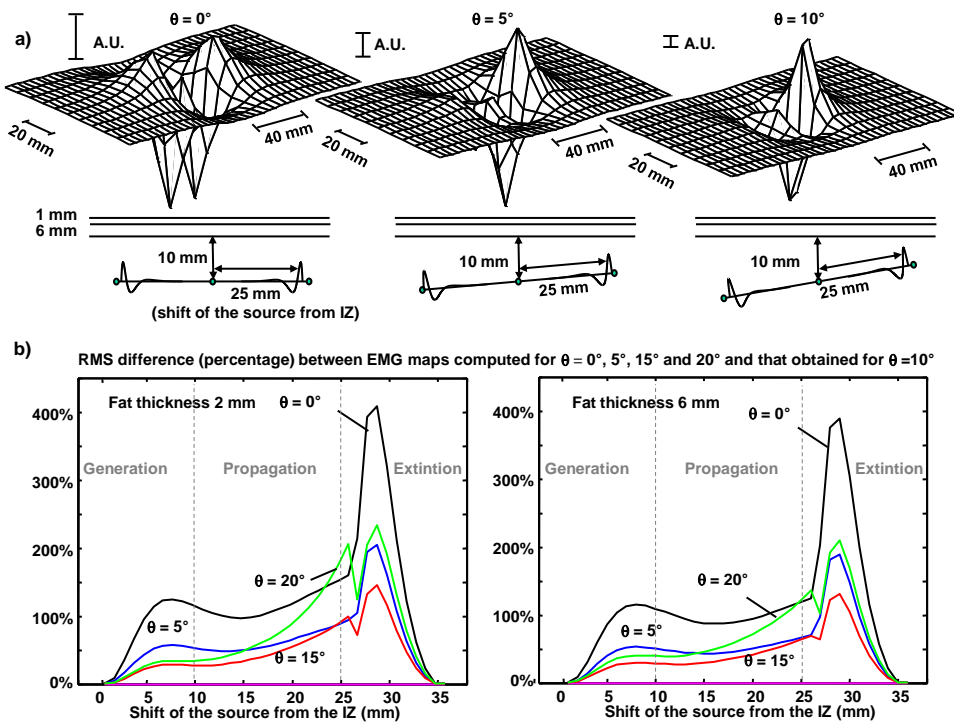
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1 Fig 1



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3 Fig 2



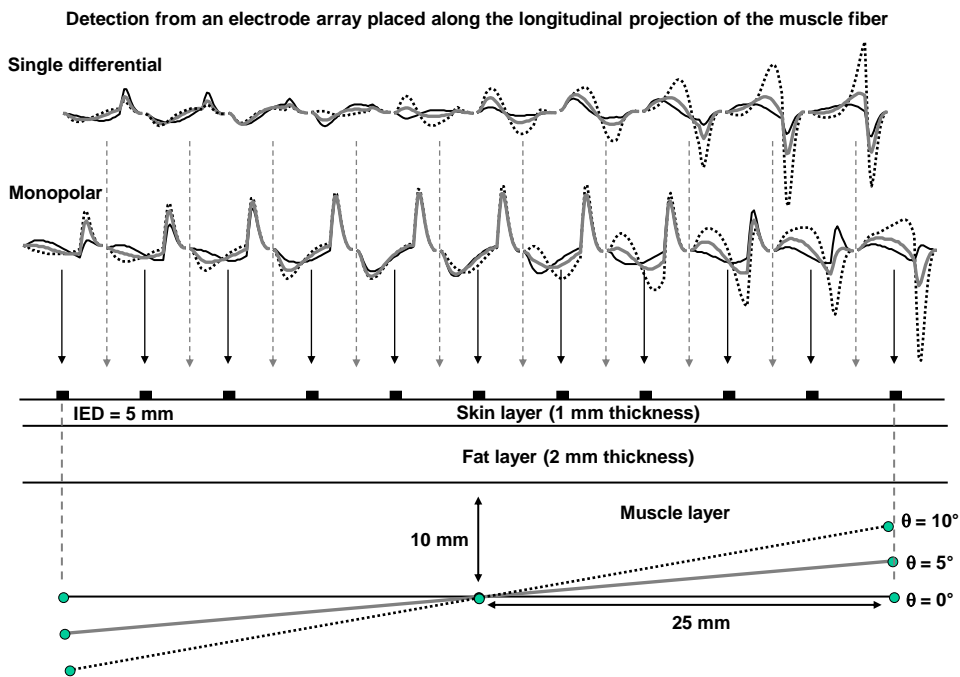
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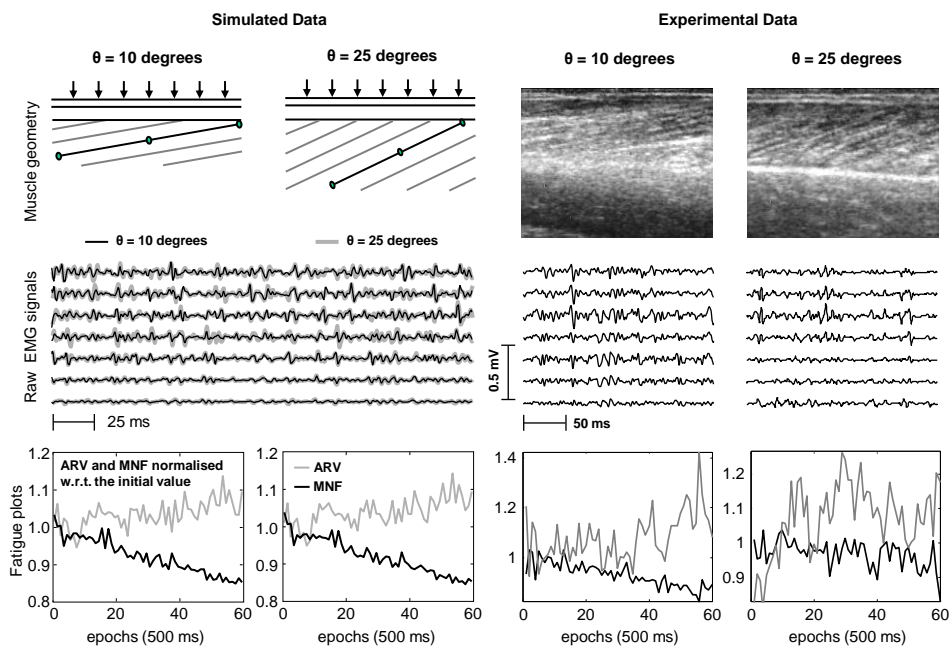
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1 Fig 3



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3 Fig 4



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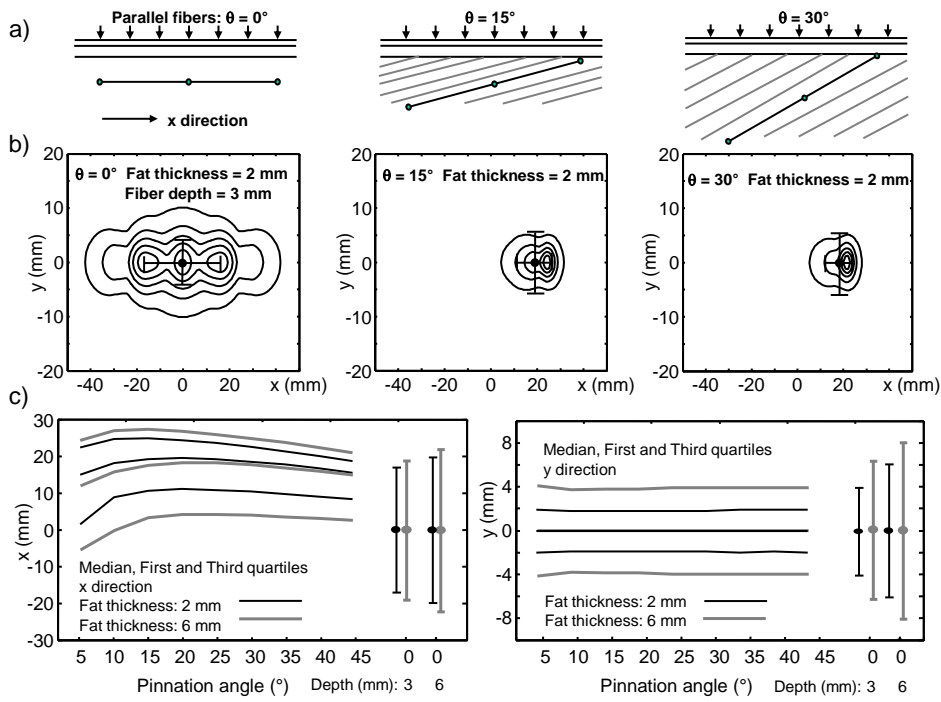
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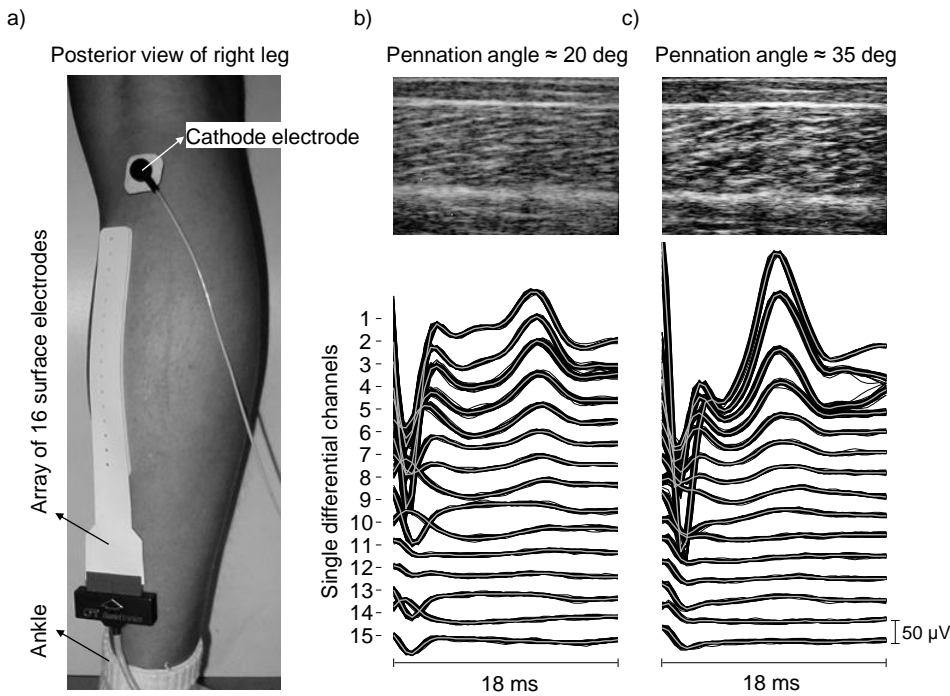
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2 Fig 5



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4 Fig 6



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