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## Identification of muscle innervation zones using linear electrode arrays: a fundamental step to measure fibers conduction velocity

Matteo Beretta-Piccoli<sup>a,b</sup> , Corrado Cescon<sup>a</sup> , Marco Barbero<sup>a</sup> and Giuseppe D'Antona<sup>b,c</sup>

<sup>a</sup>Rehabilitation Research Laboratory, Department of Business Economics, Health and Social Care, University of Applied Sciences and Arts of Southern Switzerland, SUPSI, Manno, Switzerland; <sup>b</sup>Criams-Sport Medicine Centre Voghera, University of Pavia, Pavia, Italy; <sup>c</sup>Department of Public Health, Experimental and Forensic medicine, University of Pavia, Pavia, Italy

### ABSTRACT

Fiber conduction velocity (CV) is a parameter correlated to the physiological membrane properties of the skeletal muscle fibers. Changes in muscle fiber CV are correlated to the gradation of force and fatigue, and can be measured by means of surface electromyography EMG (sEMG). sEMG measurement of CV during muscle contractions requires the correct identification of the skeletal muscle innervation zone (IZ). In superficial muscles, IZ location can be detected using linear electrode arrays and visually identified as the point of inversion of the detected motor unit action potential (MUAP) propagation. In the present work, we present a method for the effective and fast detection of the IZ location, through the following procedures: (1) identification of the target superficial muscle considering the muscle fiber architecture; (2) electrode array selection based on interelectrode distance and number of electrodes; (3) subject training to perform submaximal isometric contractions of the target muscle/s; (4) electrode array positioning along the muscle surface to investigate the IZ position during the contractions by visual identification of MUAP patterns.

### GRAPHICAL ABSTRACT

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### KEYWORDS

Innervation zone; skeletal muscles; surface EMG; motor unit action potentials; multichannel sEMG

## 1. Introduction

Muscle fatigue is the failure to maintain a certain degree of force under maximal or submaximal sustained contractions (Vollestad, 1997). Fatigue arises at the skeletal muscle level but its origin is not exclusively muscular (i.e. peripheral) but also neural (i.e. central) (Bigland-Ritchie et al., 1978). In fact, central aspects of fatigue derives from physiological properties of the motor neurons, and insufficient drive from supraspinal sites, resulting in a decrease in motor unit (MU) discharge rate, recruitment and tension development.

Peripheral contributions to muscle fatigue reflect changes that occur locally within the muscle fiber itself (e.g. altered calcium handling at the sarcoplasmic reticulum, increased inorganic phosphate and ADP concentration) and results in the hampered execution of the descending central commands (Allen, Lamb, & Westerblad, 2008).

During sustained voluntary contractions, muscle fatigue occurs with changes in the skeletal muscle myoelectric properties that can be recorded. To this aim, the definition of indices associated to the electromyographic (EMG) signal that can be recorded from inside the muscle or applying electrodes on

the skin above the selected muscle (sEMG). In particular, sEMG recordings may reflect both central and peripheral neuromuscular properties as the amplitude and the power spectrum of the sEMG signal depends on both muscle fiber membrane properties and on the timing of MU action potential (Farina, Zagari, Gazzoni, & Merletti, 2004). In particular, during an isometric constant force contraction, biochemical and physiological changes, mainly due to reduction in intracellular pH, are reflected by a decay in muscle fibers conduction velocity (CV), as demonstrated by several studies in health and disease (Beretta-Piccoli et al., 2015, 2017; Meduri et al., 2016).

Based on the above, an estimation of the rate of change of CV mainly describes muscular fatigue in isometric contractions (Merletti & Farina, 2016).

CV can be measured from the time interval between signals detected at least in two sites by multichannel sEMG (Merletti & Farina, 2016). In ideal conditions, the delay between analog signals of identical shapes can be estimated from any reference point along the recorded trace. However, these conditions are never verified in practice (Merletti & Parker, 2004). In fact, the two detected signals are never identical due to the distribution of conduction velocities of the active MUs. How the action

potentials generate and extinct, the fibers orientation with respect to the recording electrode, and/or the heterogeneity of subcutaneous fat layer also contribute to shape changes of the signals during propagation (Merletti & Parker, 2004). In particular, an overestimation of CV occurs when the signal approaches the innervation zone (IZ) and the tendon region of the muscle due to non-travelling components (end of fibers effect). Therefore, to minimize these biases, detection of the signal should be obtained between the tendon region and the IZ. Therefore, the correct identification of the anatomical localization of the IZ in the muscle of interest is of crucial importance to correctly proceed with sEMG estimation of CV and its changes due to contractions and fatigue.

An IZ is a portion of the skeletal muscle with bandlike shape in which neuromuscular junctions (NMJ) thicken (Nishimune & Shigemoto, 2018).

Different techniques have been used to detect the skeletal muscles IZ. Some of them identifies the neuromuscular structures including the Ach receptors on the post synaptic elements, and others exploit the electrophysiological properties of the neuromuscular innervations (sEMG).

Anatomical localization of NMJ allows the identification of clustered IZs of the fascicles that form the IZ in a band-like area (Kaymak, Kara, Yağiz On, Soyly, & Özçakar, 2018). The shape of this area depends from the 3D architecture of the fibers. For example, the biceps brachii muscle, frequently investigated by using sEMG (Beretta-Piccoli et al., 2017; Meduri et al., 2016; Negro et al., 2018, 2019), contains curvilinear fibers and the IZ appears as a reverse V-shaped band (5–10 mm × 4–6 cm) and the structure is located in the middle between proximal and distal myotendinous junctions. When the muscle has fascicles formed by serially connected fibers a band-like IZ is not found, but NMJ appear scattered (i.e. gracilis or sartorius muscle). When the skeletal muscle displays compartments with different structural features, each of them shows distinct band-like IZ if the fascicles are composed by fibers spanning through (i.e. semitendinosus muscle) (Kaymak et al., 2018).

When skeletal muscles are superficial, IZ location can be studied by using multichannel sEMG (Beretta Piccoli et al., 2014). Electrode array (linear or two-dimensional) is placed over the skin over the muscle of interest. Instead, IZs of the deep compartments cannot be studied by using this technique. sEMG detection can be done with monopolar electrodes which detect electrical signals generated in the end plate regions and spread towards the ends of the fibers in opposite directions. The amplified difference of two monopolar signals detected by a pair of

electrodes is called as differential EMG signal. When IZ is in the bulk muscle between two consecutive electrodes, even when correctly placed along the direction of the muscle, the amplitude of signal generated becomes significantly smaller due to the cancellation of the action potentials spreading in opposite directions. Therefore, due to these constraints, perfect positioning of surface electrodes is mandatory to identify IZs location.

In the present work we present a method to correctly detect IZs in superficial muscles in order to facilitate subsequent measurement of CV and contraction induced changes both in healthy and diseased states.

### 1.1. Muscle selection

Three aspects are fundamental in selecting the muscles for the IZ identification: muscle depth, muscle architecture, and subcutaneous tissue layers' thickness. The muscles suitable are superficial as the electrical function of deep muscles is not detectable by sEMG, since the detection volume of a pair of electrodes in bipolar configuration is approximately 10–20 mm (Farina, Cescon, & Merletti, 2002)

In addition, fusiform muscles with fibers running parallel to the skin (biceps brachii and trapezius), are suitable for motor unit action potentials (MUAPs) identification and thus identification of the IZ. On the other hand, pinnate muscles with fibers inclined with respect to the skin (gastrocnemius and biceps femoris) produce sEMG that are more difficult to interpret, with no propagation of the signals along the electrodes and no visible IZ. Lastly, the subcutaneous tissue layer thickness can affect the quality of sEMG of superficial muscles because it acts like a band pass filter that dramatically reduces the amplitude of MUAPs with respect to the background noise or power line interference.

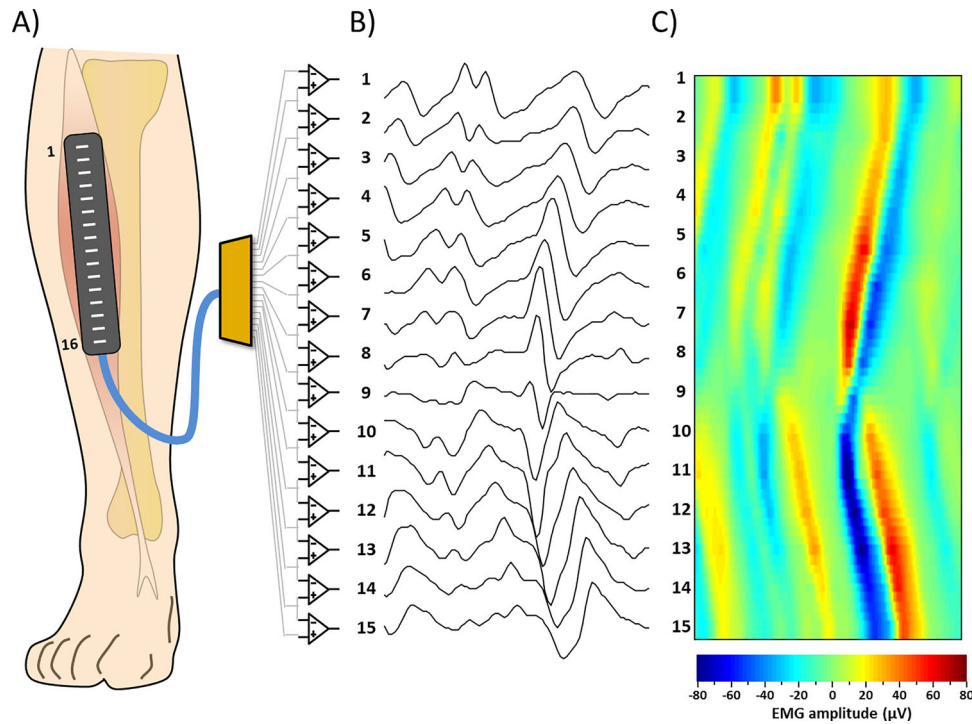
### 1.2. Electrode selection

A proper electrode array should be selected considering the muscle size. Two parameters have to be selected: the number of electrodes and the inter-electrode distance (IED).

The number of electrodes should be large enough in order to have the possibility to observe MUAPs propagation in both directions of the fibers with respect to the innervation zone, thus at least eight electrodes are necessary in the array.

Commercially available electrode arrays have usually 16 electrodes, because the original acquisition boards had 16 channels.

The IED depends on the subcutaneous tissue thickness: in case of muscle with thick subcutaneous layers (e.g. 5–10 mm as in biceps brachii or upper



**Figure 1.** Examples of sEMG signal detection. (A) Representation of the array of 16 electrodes positioned on the Tibialis Anterior muscle (B) 15 single differential signals obtained by the corresponding instrumentation amplifiers. (C) sEMG signals or the same epoch of panel B represented as an interpolated color map.

trapezius muscles) it is better to have 10 mm IED, while in case of thin subcutaneous tissues (e.g. 1–2 mm as in abductor pollicis brevis or first dorsal interosseous muscles) it is suggested to use higher spatial resolution (2.5 mm).

It is important to consider that the spatial resolution correspond to half of the IED, thus the operator should decide the optimal IED according to the specific aim of the investigation.

In our study we used a linear array of 16 electrodes in single differential configuration (Figure 1(A)). The array had an IED of 10 or 5 mm (silver bars, 5 mm long, 1 mm diameter), or 2.5 mm (silver pins, 1 mm long, 1 mm diameter) (OT-Bioelettronica, Turin, Italy).

Operator and subject were electrically grounded using reference electrodes applied to wrist or ankle.

### 1.3. Instructions to the subject

To perform EMG acquisition it is suggested to position the subject in order to facilitate the contraction of the selected muscle and place the array over the muscle belly with the electrode array aligned to the muscle fibers. Then, the subject should be instructed to activate the target muscle reducing the activation of adjacent muscles. During recording a constant force isometric contraction is necessary.

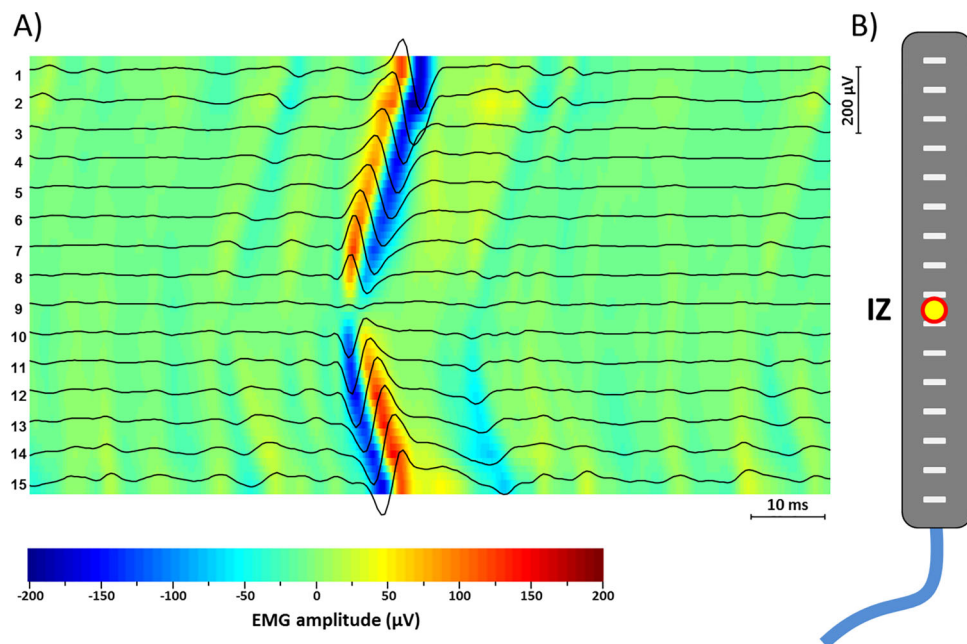
### 1.4. Signals detection and visualization

An array of instrumentation amplifiers with high common mode rejection ratio (CMRR >80dB), which

describes the amplifier capability to reject common mode voltages, and low input noise ( $<1\mu\text{V RMS}$ ) has to be used to amplify the EMG signals. The signals have to be displayed on the screen of a PC in a bi-dimensional representation with time on the x axis and channels on the y-axis. The suggested time scale to see proper MUAP shapes during a contraction is to show epochs of 50 or 100 ms, with a refresh rate of 1 s (thus in real time only 1/10 or 1/20 of the signals is observed) with EMG amplitude normalized with respect to the maximum value of the signals during the epoch (Figure 1(B)). The signals have to be separated with an offset in the vertical direction in order to avoid superposition of signals from adjacent channels. A color-scale image of the signal (15 rows corresponding to the channels and 2048 columns per seconds) was superimposed to the signals. The image was interpolated of a factor 3 in the vertical direction (resulting in  $15 \times 8 = 120$  rows) for graphical reasons (Figure 1(C)). The color image superimposed to the signals allowed to identify the MUAPs as pairs of red and blue diagonal stripes (Figure 2(A)).

We used a commercially available amplifier (EMG-USB2, OT Bioelettronica, Turin, Italy) with a bandwidth of 10–750 Hz and a gain of 2,000, 5,000, or 10,000 depending on the isometric contraction level. Signals were sampled at 2048 Hz and converted using a 12 bit A/D converter. Samples were visualized during acquisition and then stored in a personal computer using customized software developed at OT Bioelettronica (OTBiolab v2.0).

Prior to electrode placement, the skin was prepared by gentle local abrasion using medical



**Figure 2.** (A) Example of sEMG signal detection with a clearly visible MUAP. The color map. Superimposed to the signals allows an immediate identification of the propagation of the signals (V-shape) and consequent identification of the innervation zone. (B) Representation of the array of 16 electrodes and the location of the innervation zone as a yellow and red circle.

abrasive paste (Everi, Spes Medica, Genova, Italy) and cleaned with water, according to the recommendations for skin preparation (Hermens, Freriks, Disselhorst-Klug, & Rau, 2000).

Positioning of the electrode arrays was performed respecting, as much as possible, the muscle fiber direction. The aim was to allow a correct visualization of MUAPs propagation during sEMG recording.

### 1.5. Submaximal isometric contraction

During the contraction, the array has to be hold in place with Velcro straps or with the hand by the operator or the subject. After each contraction (5–10 s duration) the array is moved transversally and longitudinally along the muscle. In case the signals do not allow clear visualization of the IZ the array has to be slightly rotated in order to align it to the muscle fibers.

### 1.6. Visual analysis

In our study, detection of IZ was obtained by visual search for a minimal amplitude channel and phase reversal in the single differential signal (Merletti, Farina, & Granata, 1999).

Overall, a narrow IZ between two electrodes corresponds to a signal trace with minimal amplitude. If the IZ is underneath one electrode, reversal of the phase appears in the two differential traces coming from the two pairs sharing that electrode (Rainoldi, Melchiorri, & Caruso, 2004).

In our case, once the IZ was detected, the corresponding position laterally to the linear electrodes

array was marked on the skin of the subject (Figure 2(B)).

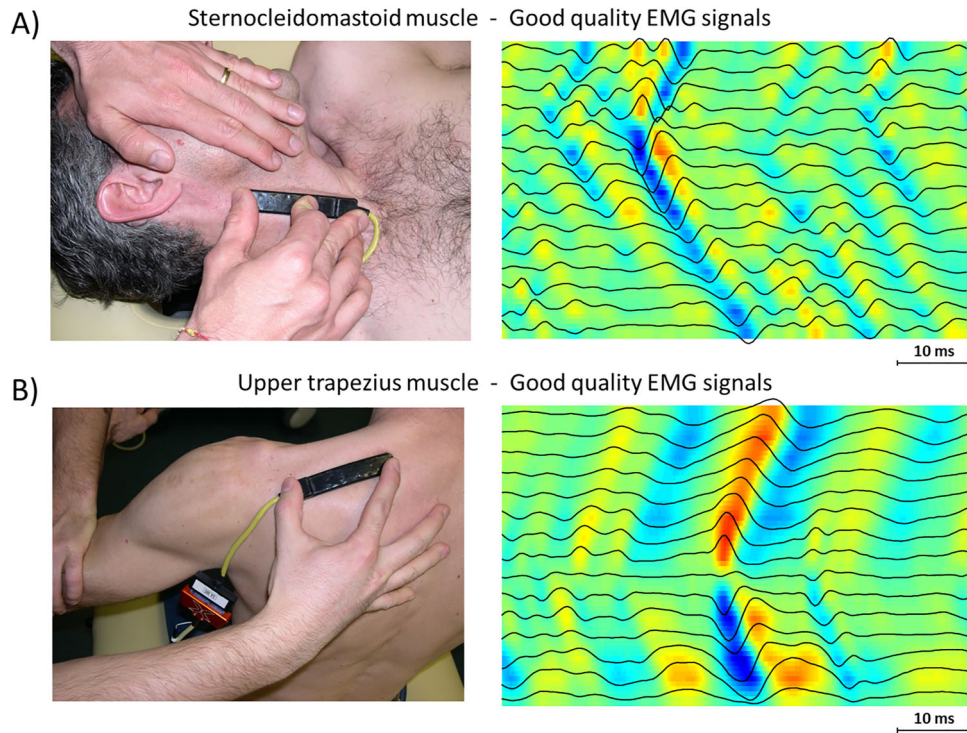
### 1.7. Examples of surface EMG signals

Figures 3 and 4 show examples of EMG signals detected from superficial skeletal muscles with fibers parallel to the skin. Figure 3 shows examples of good quality signals, where it is easy to identify the IZ location.

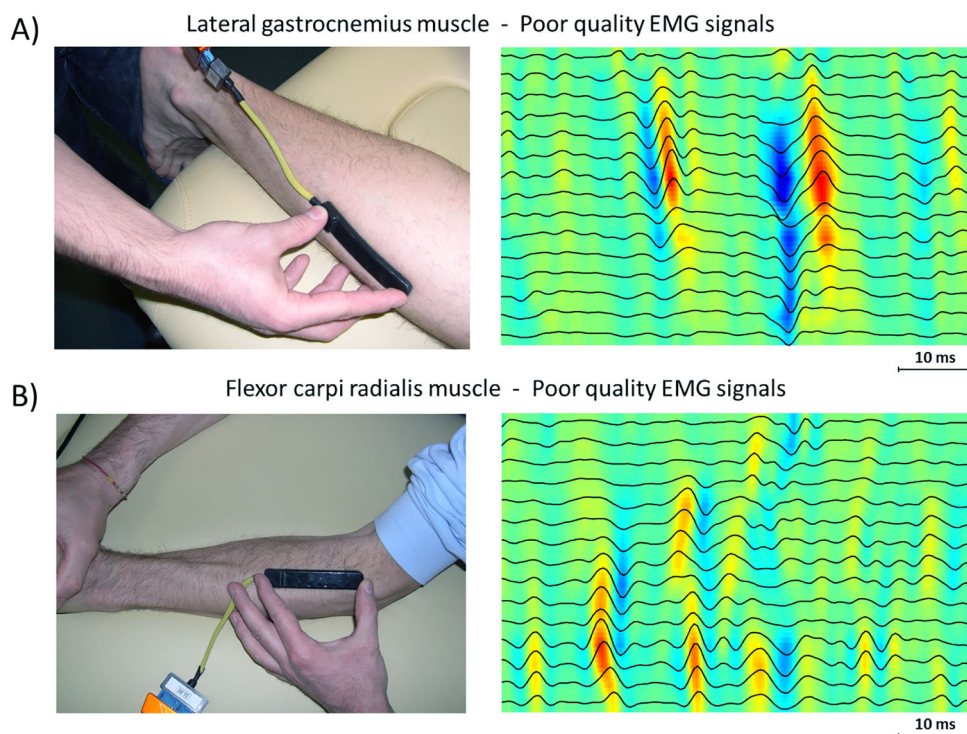
### 1.8. Clinical applications

The investigation of the IZ location has been proposed with different aims in both basic research and clinical settings: i.e. to investigate the skeletal muscle anatomy and to describe the IZ morphology (DeFreitas et al., 2010; Masuda, Miyano, & Sadoyama, 1985; Saitou, Masuda, Michikami, Kojima, & Okada, 2000; Shiraishi, Masuda, Sadoyama, & Okada, 1995). Similarly, the IZ distribution in a number of muscles has been defined to improve the electrodes placement in sEMG studies (Beretta Piccoli et al., 2014; Boccia & Rainoldi, 2014)

For the purpose of CV estimation during sEMG recordings, the effect of electrode location with respect to the tendon and IZ has been discussed by many authors (Beretta Piccoli et al., 2014; Cescon, Rebecchi, & Merletti, 2008; Nielsen, Graven-Nielsen, & Farina, 2008; Roy, De Luca, & Schneider, 1986; Ye, Beck, & Wages, 2015). It is well established that, for getting the best estimates of amplitude, spectral variables, and CV, the electrode location should be selected between the IZ and the tendon (Merletti & Parker, 2004). In fact, the IZ and tendon regions



**Figure 3.** Examples of sEMG signals of good quality from two different muscles. (A) Signal recorded from Sterno Cleido Mastoid muscle, where it is possible to recognize the IZ (between channel 4 and 5). (B) Signal recorded from upper trapezius muscle, where IZ is visible on channel 9.



**Figure 4.** Examples of sEMG signals of poor quality from two different muscles. (A) Signal recorded from Soleus muscle, where it is not possible to identify an IZ (although two MUAPs are clearly visible). (B) Signal recorded from upper Flexor Carpi Radialis muscle, where the EMG signals do not allow identification of IZ.

determine positive bias in spectral frequency estimates and negative bias in amplitude values and the estimation of CV from regions close to IZ or tendon locations may be completely wrong, due to the absence of potentials traveling in space with similar shape.

The effect of isometric contraction intensities and joint range of motion on the IZ location has been also described (DeFreitas et al., 2008, 2010; Martin & MacIsaac, 2006; Piitulainen, Rantalainen, Linnamo, Komi, & Avela, 2009). Additionally, the influence of the electrodes placement over the IZ on sEMG

variables has been extensively studied, i.e. (Beck, Housh, Cramer, & Weir, 2007; Beck et al., 2009; Nishihara et al., 2010; Rantalainen, Kłodowski, & Piitulainen, 2012). IZ detection has been also proposed to optimize the neuromuscular electrical stimulation of skeletal muscles or to inject neuromuscular blockers like the botulinum toxin (type A) (Wissel et al., 2009; Ward, 2008).

In all these studies, the IZs are generally localized according to the bony landmarks whose positions are not modified by the movement of the corresponding joint segments. However, during isometric contractions or electrical activations of the muscle, the IZs shift proximally with the flexion of certain joints (Huang et al., 2019; Nishihara, Kawai, Chiba, Kanemura, & Gomi, 2013; Piitulainen et al., 2009). This phenomenon could be explained by the muscle fibers shortening towards the fixed segment of the joint and/or to the lengthening of the distal tendon.

Knowledge about the IZ position may be also relevant to investigate neuromuscular diseases. Recently with the intent to address the myofascial trigger point physiopathology, the spatial relationship between the IZ and the trigger point has been explored (Barbero et al., 2013). Moreover, the biopsy of the IZ may help the diagnosis of different kind of myasthenia (Slater et al., 2006). Finally, surgical incisions of muscles may be guided to avoid the IZ, and consequently nerve lesions: for example, in pregnant women, in order to avoid faecal incontinence, the location of IZ of the anal sphincter should be considered prior to episiotomy (Cescon et al., 2014).

In this work we described a simple, fast and reliable method to non-invasively detect IZ in superficial muscles.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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## ORCID

Matteo Beretta-Piccoli  <http://orcid.org/0000-0003-4794-2540>

Corrado Cescon  <http://orcid.org/0000-0002-1632-6684>

Marco Barbero  <http://orcid.org/0000-0001-8579-0686>

Giuseppe D'Antona  <http://orcid.org/0000-0002-0683-5565>

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