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Consensus for experimental design in electromyography (CEDE) project: High-density surface electromyography matrix

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INTRODUCTION

High-density surface electromyography (HDsEMG) is a technique that involves the concurrent recording of at least four surface electromyographic (EMG) signals with closely spaced, small-diameter electrodes (Masuda et al., 1983; Merletti et al., 2003; Zwarts and Stegeman, 2003). By concurrently recording EMG signals from different locations over one or more muscles of interest (Figure 1), HDsEMG characterizes the spatial distribution of EMG amplitude over the skin and how it changes over time. This can be used to identify different features of the neuromuscular system such as regional activation, muscle fiber properties and single motor unit activity. Specific applications on when HDsEMG should be used instead of conventional bipolar surface EMG or intramuscular EMG recordings are described elsewhere (Besomi et al., 2020).

Regional activation is a term commonly used to describe the recruitment and modulation of motor units localized in a region of a muscle. As the regional recruitment of muscle fibers can be observed in the HDsEMG as an amplitude distribution localized above the active fibers (Rodriguez-Falces et al., 2013; Roeleveld et al., 1997; Vieira et al., 2011), local variations of surface EMG amplitude can be interpreted as variations in the activity of muscle fibers localized in different muscle regions (Holtermann et al., 2005; Madeleine et al., 2006). The association between localized motor unit recruitment and regional activation observed with HDsEMG has been described in studies using intramuscular recordings (Falla and Farina, 2008; Watanabe et al., 2012), electrical stimulation (Gallina et al., 2016), and voluntary activation (Gallina and Botter, 2013; Zhou et al., 2011).

When used to characterize how action potentials propagate along the muscle fibers, HDsEMG has been used to describe properties of the muscle fibers, such as conduction velocity (Farina et al., 2000), location of the main innervation zone (Masuda et al., 1983), location of the musculotendinous junction (Merletti et al., 2001), fiber length (Schulte et al., 2005), fiber orientation (Lapatki et al., 2005), and properties of the spatial distribution of the motor unit action potential (Vieira et al., 2011). Although several of these measures lack validation against gold standard anatomical techniques, they have been successfully used to characterize the physiology of the musculoskeletal system in health and pathology, such as altered action potential propagation in generalized myotonia (Drost et al., 2001),

altered spatial distribution of motor unit action potentials in people with stroke (Vieira et al., 2019), and increased effectiveness of botulinum toxin when injected in proximity of the muscle innervation zone (Lapatki et al., 2011).

As most motor units have a unique spatial distribution of their action potentials when recorded on the skin (Farina et al., 2008), the firing times of individual motor units can be extracted from HDsEMG (Disselhorst-Klug et al., 1999; Holobar and Zazula, 2007; Kleine et al., 2007). The derived information concerning motor unit recruitment and firing rate frequently provides a better representation of neural drive to the muscle than EMG amplitude (Farina et al., 2004; Martinez-Valdes et al., 2018) and it enables estimation of muscle fiber properties at the motor unit level (Farina et al., 2009; Lapatki et al., 2005). Decomposition algorithms for HDsEMG are currently validated for signals acquired during isometric contractions (Holobar et al., 2010).

The aim of this matrix is to review the main uses, advantages, and limitations of HDsEMG, and to provide indications on recommended and non-recommended applications of this technique. This matrix was developed by an international consensus of experts as part of the Consensus in Experimental Design in Electromyography (CEDE) Project using a Delphi process.

[Insert Figure 1 here]

METHODS

A detailed description of the project, including the method for expert group selection and the process for the development of the CEDE matrices, can be found elsewhere (Besomi et al., 2020, 2019; Hodges, 2020; McManus et al., 2021). In brief, the steering committee and the lead investigator prepared a draft of the matrix, and this was sent to the other CEDE members to reach consensus of the content following a Delphi process. Participants of the Delphi process are co-authors. The Human Research Ethics Committee of The University of Queensland, Australia provided ethical approval for this project.

Development of the draft

The steering committee (CDK, DF, RM) and the lead investigator (AG) prepared a first draft of the matrix. Cells of the matrix were organized according to three most common applications of HDsEMG: 1) regional activation, 2) muscle fiber properties, and 3) single motor unit activity. For each application, content was arranged into five sections: a) electrode montage; b) electrode type and configuration; c) electrode location and orientation; d) data analysis; and e) interpretation. Based on relevance, each section included one or more of the following sub-sections: general considerations, pros, cons, caution, recommended use, non-recommended use, and a summary of information to report.

Delphi process

The process followed that of other CEDE projects (Besomi et al., 2020, 2019; McManus et al., 2021). The Delphi process is a widely accepted method to achieve consensus and is used as a decision-making method (Waggoner et al., 2016). In the first round, 18 members of the CEDE team were invited to review the matrix and provide feedback. Four members reported that they did not wish to participate in this specific CEDE project because it was not within the scope of their expertise. The criteria to obtain consensus are described in other matrices of the CEDE project (Besomi et al., 2020, 2019; McManus et al., 2021). The steering committee, the lead investigator, and the coordinator (MB) oversaw the project and integrated comments but did not participate in the Delphi process. The Delphi questionnaires were sent online using a centrally supported survey tool from the University of Queensland (i.e., Checkbox). All data were entered and processed with Microsoft Excel ®. For each item, we rated the percentage of participants rating each outcome as appropriate (score 7–9), uncertain (score 4–6) and inappropriate (score 1–3) and calculated the median and interquartile range (IQR).

RESULTS

From the 14 experts who agreed to participate in the Delphi process, 13 (93%) replied to the first-round questionnaire. Version 1 was composed of 89 items. After round one, 15 sections were ranked with insufficient consensus. For round two, the 15 sections were resubmitted to the entire group. Fourteen experts (100%) completed the second-round questionnaire. Two sections were still ranked

with insufficient consensus (IQR = 2.3) and, because comments were minor, the integrated version of these items was sent only to the contributors that rated the item lower than 7 points for their endorsement. A summary of the results of the Delphi consensus process is presented in Appendix 1. The final HDsEMG matrix endorsed by the CEDE project team is presented in Table 1.

[Insert table here]

DISCUSSION

The matrix developed in this Delphi consensus project presents a summary of recommendations on the use of HDsEMG. We focused on three most common applications: the estimation of regional muscle activation, the characterization of muscle fiber properties, and the identification of single motor unit activities. Strengths and limitations of this consensus process have been described in detail elsewhere (Besomi et al., 2019). Where possible, we gathered evidence from experimental studies in humans, and when these were not available, we based our recommendations on simulations or theoretical considerations. This matrix will be updated when new experimental data become available. The information contained in this matrix does not replace formal training or education in the application and interpretation of HDsEMG.

This matrix demonstrates the wealth of information that can be extracted from HDsEMG in comparison to conventional bipolar electrodes. Although information regarding regional activation, muscle fiber characteristics and single motor unit activity may appear straightforward to obtain from HDsEMG recordings with currently available algorithms, correct use of the technique depends on careful consideration of several steps. First, when planning an investigation focused on one of the applications above, one should consider whether HDsEMG is the most appropriate technique to obtain the information needed. Other techniques (Besomi et al., 2019), including anatomical or histological approaches may be more appropriate. Second, once it is established that HDsEMG is the most appropriate technique to obtain the information needed, many aspects of the application require careful planning. For instance, the size, inter-electrode distance and position of the array should be considered, and selections made in accordance with both the research question and the characteristics of the muscle

that is under investigation (e.g., muscle architecture - fusiform vs. pennate). Third, the limitations of the technique should be considered and acknowledged. As noted in the matrix presented here, these limitations vary across applications. They may include an absence of means to establish validity or reliability, and selective sampling of signals generated by superficial motor units. If these steps and the other recommendations in the matrix are followed, HDsEMG can provide unique information about the neural drive to the muscle, neuromuscular activation and muscle fiber characteristics that cannot be obtained with any other experimental techniques currently available.

Discussion during the Delphi process highlighted several key issues related to HDsEMG. First, the validity of some features extracted from HDsEMG, specifically the location of the innervation zone and the dynamics of the spatial distribution of the motor unit action potential. This highlights the need for validation studies, that employ HDsEMG paired with other techniques that can provide an accurate measure of the physiological process or anatomical feature of interest. Second, an issue for discussion was the necessity for caution when inferring regionally specific muscle activation, as variations observed via HDsEMG may be due to anatomical factors rather than preferential neural drive to a muscle region, especially during non-isometric contractions. Third, the group discussed that there are several issues that are often not acknowledged in HDsEMG studies, including the potential presence of crosstalk in the recordings and the absence of standardized procedures to normalize the HDsEMG amplitude signals (Besomi *et al*., 2020).

Many of the studies considered to create this matrix focused on motor unit identification, conduction velocity, location of the innervation zone and regional activation. In contrast, the investigation of other muscle fiber characteristics is limited to only a few studies, and generally without data regarding validity and reliability. There is a need to generate additional empirical data to determine whether these estimates can be used to describe the characteristics of the muscle of interest.

CONCLUSION

HDsEMG can provide a wealth of information about the neuromuscular system . This matrix details the recommendations of members of the CEDE team regarding the manner in which HDsEMG can be used to obtain information on regional activation, muscle fiber properties and single motor unit activity. This matrix is intended to help HDsEMG users when collecting, reporting, and interpreting data, and is not an exhaustive guide that can replace formal training or education. We hope that this matrix will prompt discussion regarding the use of HDsEMG and will stimulate researchers to generate new empirical data to update this matrix, with the ultimate goal of furthering our understanding of the human neuromuscular system in health and disease.

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Declaration of Competing Interest

Dario Farina is a scientific advisor for the company OT Bioelettronica, Torino, Italy, and for Facebook Reality Labs, NY, US. No other known competing financial interests or personal relationships are reported.

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TABLE 1. High-density surface EMG matrix.

Definition	High-density surface electromyography (HDsEMG) is a technique that involves the concurrent recording of at least 4 surface electromyographic (sEMG) signals with closely spaced (normally 2.5 – 10 mm), small-diameter (0.5 – 3mm) electrodes.
General considerations	*Purpose of HDsEMG:* HDsEMG is used to measure the spatial distribution of the potentials associated with the generation and propagation of action potentials along muscle fibers. By having 4 to several hundred surface electrodes placed in a known arrangement on the skin over a muscle or muscle group, HDsEMG provides information about the temporal and spatial features of muscle activation. The signals can provide information on regional activation, muscle fiber properties, and single motor unit activity. *Sampling rate:* As the bandwidth of signals collected with HDsEMG is approximately 10-500 Hz, a sampling rate of 1000- 2000 Hz is commonly used to collect these signals. A sampling rate of at least 2000 Hz is recommended to represent action potential shapes without the need for interpolation. *HDsEMG detection systems:* Electrodes can be arranged in linear or bi-dimensional arrays. Linear arrays are used to detect the spatial distribution of surface electromyographic (sEMG) amplitude in a single dimension, while bi-dimensional arrays allow the assessment of the spatial distribution of the electromyographic signal over the skin surface. *Electrode size and spacing:* Small diameter (normally in the range of 0.5-3mm) electrodes are necessary to reduce the spatial low-pass filtering effect on the distribution of electric potentials on the skin, which is averaged under the electrode area. Similarly, the distance between electrodes should be small (normally up to 10 mm) to increase the spatial resolution and to avoid spatial aliasing due to spatial under-sampling of the action potential distribution on the skin due to large inter-electrode distance; see (Merletti and Muceli, 2019) for details. *Spatial filtering:* HDsEMG is usually recorded in monopolar montage, meaning that variations of potential on the skin are detected from each electrode of the array with respect to a common reference electrode. The detection volume of the sEMG recording, as well as the presence of propagating and non-propagating components, can be manipulated online or off-line by spatial filtering. This involves computing the weighted sum of monopolar sEMG recordings collected by electrodes in spatially defined locations. This processing can only be applied off-line if the amplifiers used to record the monopolar signals have identical characteristics (gain, phase); otherwise, spatial filters can be implemented online by hardware, which allows collection of signals directly in the chosen electrode montage. The most commonly used spatial filter is the single differential (difference between a pair of electrodes; weighting +1 and -1; also known as bipolar), followed by higher order filters such as double differential (3 electrodes in a line; weighting 1; -2; 1) and the two-dimensional Laplacian filter (5 electrodes arranged crosswise, with the central one having a weight of -4 and the peripheral ones having weight of 1). In general, spatial filters with more electrodes reduce the detection volume (more selective) and decrease the presence of non–propagating components such as power line interference, action potential generation and end-of-fiber effect (associated with the extinction of the action potential). One-dimensional spatial filters (single and double differential) require constant inter-electrode distance along the direction the spatial filter is applied in; bi-dimensional spatial filters (Laplacian) require equal inter-electrode distance along both dimensions. In muscles with fibers parallel to the skin, single and double differential filters should be applied to signals collected from electrodes placed along the muscle fiber

	direction; this is not possible in muscles with pennate architecture in depth direction. It should be noted that both the temporal shape and the spatial distribution of the spatially filtered action potential depend on the electrode montage. *Hardware specifications:* Amplifiers for HDsEMG must have identical gains and phase shifts; in addition, they must have one A/D converter per channel, or a fast multiplexer, or software compensation of the multiplexer delay. This is especially relevant when spatial filters are applied off-line (by software). Due to the small electrode diameter and the associated high contact impedance, the pre-amplifiers must have a high input impedance in order to reduce the power line interference due to different electro-skin impedances. Active electrodes (connected directly to the pre-amplifiers) are recommended to eliminate the risks of artifacts due to movements of the cables between the electrodes and the pre-amplifier. For more details, see (Merletti and Cerone, 2020; Merletti and Muceli, 2019). *Data quality assessment:* Besides the data quality assessment generally performed in traditional bipolar and intramuscular electromyography techniques, which includes evaluation of the presence of power line interference, artifacts, and noise, HDsEMG offers additional ways to ensure that the sEMG recordings reflect physiological information. It is good practice to ensure that features expected from the specific anatomy of the muscle being tested (such as presence or absence of action potential propagation in muscles with fibers parallel to the skin or pennate architecture in depth direction respectively, presence of innervation zones, fiber orientation) can be observed in the HDsEMG signals.		
Application of HDsEMG	*1) Regional activation*	*2) Muscle fiber properties*	*3) Single motor unit activity*
Definitions	Identification of the electrical potential generated by motor units localized in different regions within a muscle, or by different muscles if the HDsEMG electrodes are placed over a muscle group. Common parameters include the location, the size, and the magnitude of the active region.	Estimation of properties of the muscle fibers. These properties are unrelated to the estimation of neuromuscular activation patterns, and include: average muscle fiber conduction velocity, location of the main innervation zone, location of muscle-tendon regions, fiber orientation on the plane of the skin, length of muscle fibers, location of muscle fibers innervated by a single motoneuron (in conjunction with single motor unit analysis).	Identification of the firing pattern of several superficially located motor units at varying force levels. Observation of the firing pattern of relatively large groups of superficially located motor units (population) may be possible in some muscles.
Examples of applications for the assessment of neuromuscular function in health and pathology	- Chronic and acute pain affect the regional activation within a muscle. - Biofeedback techniques can be used to facilitate redistribution of activity between regions of a muscle during a task. - Fasciculation potentials occurring in different muscle regions can be observed using HDsEMG.	- Average muscle fiber conduction velocity decreases during fatiguing contraction due to changes in ionic concentrations. - Average muscle fiber conduction velocity in single motor units is lower in patients with muscular disorders like Duchenne muscle dystrophy or channelopathies.	- Motor unit firing rate and recruitment are affected by fatigue. - Motor unit firing rate is modified in patients suffering from disorders such as Stroke or Cerebral Palsy. - Motor unit recruitment is different in patients with spinal muscle atrophy.

	- Changes in the spatial distribution of surface EMG amplitude occur during isometric and non-isometric fatiguing contractions in healthy individuals.	- Action potential propagation is blocked during transient paresis in patients with generalized myotonia. - Muscle fibers innervated by a single motoneuron are less localized within the medial gastrocnemius after stroke. - Botulinum neurotoxin results in larger reduction of compound muscle action potential if injected in proximity of the innervation zone.	- Motor unit firing rate is modified in different ways depending on the type of exercise intervention.
Tasks or experimental condition	- Isometric contractions. - Non-isometric contractions (caution generally required because of changes and movement of the muscle fibers relative to the skin). - Evoked potentials (such as muscle/nerve stimulation, H-reflexes, transcranial magnetic stimulation).	- Isometric contractions. - Non-isometric contractions (caution generally required because of changes and movement of the muscle fibers relative to the skin). - Evoked potentials (such as muscle/nerve stimulation). - In combination with single motor unit recording to obtain motor unit fiber characteristics.	- Isometric contractions. - Non-isometric contractions (currently under development). - Evoked potentials (generally limited to techniques that elicit responses of motor units already recruited during a voluntary contraction).
a) Electrode montage			
General considerations [##] note: throughout the document, it is assumed that recordings from "muscles with fibers parallel to the skin" are obtained from several electrodes placed along the muscle fiber direction. Muscles with pennate architecture in a	<u>Monopolar:</u> - Muscles with fibers parallel to the skin [#]: the sEMG spatial amplitude distribution consists of high-amplitude values above the innervation zone, and a gradual decrease in amplitude along the muscle fiber direction. - Muscles with pennate architecture in depth direction [##]: high-amplitude values are observed above the location of the active muscle fibers, where the fibers are closest to the skin. <u>Single Differential:</u> - Muscles with fibers parallel to the skin: the sEMG spatial amplitude distribution shows low-amplitude	<u>Monopolar:</u> - Muscles with fibers parallel to the skin: monopolar recordings consist mainly of large non-propagating components resulting from generation and extinction of the action potential along the muscle fiber. Action potential propagation can be observed in M-waves and in the spike-triggered average of single motor unit firings (see Data analysis – Single motor unit activity). The polarity of the action potential is the same on the two sides of the innervation zone. - Muscles with pennate architecture in depth direction: neither propagation nor innervation zones can be observed. <u>Single Differential:</u>	<u>Monopolar:</u> - Muscles with fibers parallel to the skin: the spatial distribution of single motor unit action potentials generally spans many channels. It is highly likely that different motor units cannot be distinguished when assessed visually from the multiunit signal. - Muscles with pennate architecture in depth direction: the spatial distribution of single motor unit action potentials generally spans several channels (less than in muscles with fibers parallel to the skin). In the multiunit signal, different motor units may appear similar when assessed visually. Motor unit action potential amplitude is larger above the fiber region closest to the skin, and it is smaller above the fiber region further away from the skin.

plane parallel to the skin (e.g., vastus medialis, pectoralis major) are considered to be “muscles with fibers parallel to the skin”. [##] note: throughout the document, “pennate architecture in depth direction” refers to muscles with large pennation angles in the depth direction (e.g., gastrocnemius medialis). Smaller (10-15 degrees) pennation angles will result in recordings more similar to muscles with fibers parallel to the skin.	values above the innervation zone, and high-amplitude values along the muscle fiber direction. - Muscles with pennate architecture in depth direction: high-amplitude values are observed above the location of the active muscle fibers, where the fibers are closest to the skin. <u>Double Differential and Laplacian:</u> - Muscles with fibers parallel to the skin: the sEMG spatial amplitude distribution usually consists of high-amplitude values above the innervation zone, and high-amplitude values along the muscle fiber direction (although further experimental research is needed to confirm these findings). - Muscles with pennate architecture in depth direction: high-amplitude values are observed above the location of the active muscle fibers, where the fibers are closest to the skin	- Muscles with fibers parallel to the skin: propagation can be observed as action potentials with similar shape in different channels. The polarity of the detected propagating potentials is reversed above the location of the innervation zone, where one or few channels with low sEMG amplitude can be observed. In consecutive channels between the innervation zone and the tendon, the action potentials should appear with similar shape but delayed in time because of the propagation of the action potential along the fibers under the electrodes. Misalignment between the muscle fiber direction and the electrode orientation (both in depth and on the plane of the skin) results in an uneven amplitude of the action potential as observed along the array/grid, with larger potentials observed above the fiber region closest to the electrodes. Propagation is not seen above the tendon region. The potentials recorded in this region are largely synchronous. - Muscles with pennate architecture in depth direction: neither propagation nor innervation zones can be observed. <u>Double Differential and Laplacian:</u> - Muscles with fibers parallel to the skin: propagation can be observed as action potentials with similar shape in different channels. The polarity of these action potentials is the same on the two sides of the innervation zone, identified as a channel with amplitude higher than the neighboring ones. Between the innervation zone and the tendon, the action potentials appear with a progressive delay because of the propagation of the action potential along the fibers under the electrodes. Almost fully-synchronized signals (i.e., delay close to zero) observed between channels positioned above the tendon region. - Muscles with pennate architecture in depth direction: neither propagation nor innervation zones can be observed.	<u>Single Differential:</u> - Muscles with fibers parallel to the skin: the spatial distribution of single motor unit action potentials generally spans several channels. During very low-force contractions, different motor units may be distinguished in the multiunit signal when assessed visually. - Muscles with pennate architecture in depth direction: the spatial distribution of single motor unit action potentials generally spans only a few channels because the distance between fibers and electrodes increases with fiber depth. During very low-force contractions, different motor units may be distinguished in the multiunit signal when assessed visually. <u>Double Differential and Laplacian:</u> - Muscles with fibers parallel to the skin: the spatial distribution of single motor unit action potentials generally spans some channels. Different motor units may be distinguished when assessed visually. - Muscles with pennate architecture in depth direction: the spatial distribution of single motor unit action potentials generally spans only a few channels because the distance between fibers and electrodes increases with fiber depth. Different motor units may be distinguished when assessed visually.

Pros *can be pros or cons, depending on the application	*Monopolar:* - Allows the detection of non-propagating components. This is useful to determine generation and end-of-fiber effects.* - Allows the selection of which spatial filter should be used after data collection, albeit with poorer rejection of common mode interference than if this processing had been completed in hardware. - Large detection volume, independently from inter-electrode distance.* - Is the preferred montage if the inter-electrode distance is not fixed (e.g., electrodes mounted on elastic textile support). - Alignment of the electrodes with respect to the fiber orientation does not influence the characteristics of the sEMG signals (e.g., fan-shaped muscles such as vastus medialis or pectoralis major). *Single Differential:* - Reduces the amount of non-propagating components.* This is useful to determine the location of the active muscle fibers. - Reduces power line interference, ECG artifacts and crosstalk. - Smaller detection volume than monopolar recordings.* for single differential recordings, smaller inter-electrode distances result in smaller detection volume. *Double Differential and Laplacian:* - Substantially reduces the amount of non-propagating components.* This is useful to determine the location of the active muscle fibers. - Substantially reduces power line interference. ECG artifacts and crosstalk. - Smaller detection volume than monopolar and single differential recordings.* When double	*Monopolar:* - Allows the detection of non-propagating components.* This is useful to determine generation and end-of-fiber effects. - Allows the selection of which spatial filter should be used after data collection. - Allows the detection of the original shape of the motor unit action potentials without any information loss due to spatial filtering. *Single Differential:* - Reduces the amount of non-propagating components.* This is useful to accurately determine the propagation velocity of the action potential along the muscle fiber. - The location of the innervation zone can be identified as an inversion of the polarity of the action potential. - Absence of delay between action potential in consecutive channels allows determining the location of muscle-tendon region. - Reduces power line interference and ECG artifacts. - Smaller detection volume than monopolar recordings.* *Double Differential and Laplacian:* - Substantially reduces the amount of non-propagating components.* This is useful to accurately determine the propagation velocity of the action potential along the muscle fibers. - Substantially reduces power line interference and ECG artifacts.	*Monopolar:* - Allows the selection of which spatial filter should be used after data collection. *Single Differential:* - Some decomposition algorithms require the application of spatial filters to identify the timing of motor unit firings. *Double Differential and Laplacian:* - Some decomposition algorithms benefit from the application of spatial filters to identify the timing of motor unit firings.

	differential signals are computed on consecutive channels, smaller inter-electrode distances result in smaller detection volume.	- Smaller detection volume than monopolar or single differential recordings.*	
Cons *can be pros or cons, depending on the application	*Monopolar:* - Contamination by power line interference and stimulation artifacts more likely than when spatial filters are used. - Contamination by ECG artifact, especially in trunk muscles, more likely than when spatial filters are used. - Contamination by crosstalk more likely than when spatial filters are used. *Single Differential:* - When considering a series of single differentials, misalignment of the electrodes in an array with respect to the fiber orientation results in progressively lower amplitude of the sEMG signals as the distance between fibers and electrodes increases. - Application of other spatial filters is difficult except for higher-order differential filters (such as the double differential). *Double Differential and Laplacian:* - When considering a series of double differential or Laplacian signals, misalignment of the electrodes with respect to the fiber orientation results in progressively lower amplitude of the sEMG signals as the distance between fibers and electrodes increases. - Application of other spatial filters (e.g., single differential) is not possible.	*Monopolar:* - Contamination by power line interference and stimulation artifacts more likely than when spatial filters are used. - Contamination by ECG artifact, especially in trunk muscles, more likely than when spatial filters are used. - Contamination by crosstalk more likely than when spatial filters are used. *Single Differential:* - Single differential detection changes the temporal shape of the motor unit action potential (approximates a differentiation).* - Application of other spatial filters is difficult except for double differential. *Double Differential and Laplacian:* - High-order spatial filters change the temporal shape of the motor unit action potential.* - Application of other spatial filters (e.g., single differential) is not possible.	*Monopolar:* - Does not allow use of all decomposition algorithms. *Single Differential:* - The spatial distribution of sEMG amplitude associated with individual motor units (e.g., moto unit action potentials) cannot be obtained in monopolar montage. *Double Differential and Laplacian:* - The spatial distribution of sEMG amplitude associated with individual motor units cannot be obtained in monopolar or single differential montage.
Recommended use	- Data should be collected in monopolar montage to have the option to analyze the data in monopolar montage or to apply spatial filters. - Monopolar montage should be used if the spatial distribution of the action potential generation or end-of-fiber effect are of interest.	- Data should be collected in monopolar montage to have the option to analyze the data in monopolar montage or to apply spatial filters. Exception: If a dry repositionable linear array is used to search for the innervation zone and/or the approximate fiber orientation, single differential signals (obtained online	- Data should be collected in monopolar montage to have the option to apply motor unit decomposition algorithms on monopolar signals or after spatial filtering. - Spatial filters should be applied according to the decomposition method chosen.

	- Spatial filters should be used if the spatial distribution of the action potential along the muscle fiber orientation is of interest. - Spatial filters should be used when recordings from more superficial regions of the muscle are of interest.	via software or by hardware) are recommended. More selective filters (double differential signals) may be needed to identify fiber orientation at higher force levels. - Single differential signals should be used to identify the approximate muscle fiber orientation using a dry array. Monopolar montage is generally used to determine muscle fiber orientation if HDsEMG is combined with M-waves or spike-triggered average of single motor unit firings (see Data analysis – Single motor unit activity). - Single differential signals should be used to identify the location of the innervation zone and of the muscle-tendon region. - Double Differential signals are recommended to estimate average muscle fiber conduction velocity.	
Non-recommended use	- Monopolar montage should not be used if the sEMG signals display significant power line interference, ECG artifact or crosstalk from the activation of surrounding muscles. - Spatial filters should be used with caution when muscle fiber orientation and pennation differs between muscle regions (i.e.: if some electrodes are aligned with the muscle fiber direction, and others are not).	- Monopolar montage should not be used if the sEMG signals display significant power line interference, ECG artifact or crosstalk from the activation of surrounding muscles. - Monopolar or single differential montages should not be used directly to estimate average muscle fiber conduction velocity.	- Monopolar montage should not be used for decomposition algorithms requiring spatially-filtered sEMG signals. If signals are collected in monopolar montage, single or double differentials should be calculated offline before applying the algorithms.
To report	- Electrode material, type and size, number of electrodes, spatial organization, inter-electrode distance. - Electrode montage used for data collection and for data analysis. - If spatial filters are applied, report which configuration and which electrodes were used.	- Electrode material, type and size, number of electrodes, spatial organization, inter-electrode distance. - Electrode montage used for data collection and for data analysis. - If spatial filters are applied, report which configuration and which electrodes were used.	- Electrode material, type and size, number of electrodes, spatial organization, inter-electrode distance. - Electrode montage used for data collection.
b) Electrode type and configuration			
General considerations	- The HDsEMG type, size and inter-electrode distance should be decided according to the size of the muscle (or muscle group) of interest, the spatial	- The HDsEMG type, size and electrode density and the inter-electrode distance should be decided according to the specific application and subsequent	- Larger arrays with smaller inter-electrode distances usually allow a better discrimination of action potentials.

	resolution needed, and the specifications of the hardware (number of channels available).	processing planned. Small inter-electrode distances (≤10mm) are generally required.	
Cautions	- If different spatial resolution is needed in the proximal-distal and medial-lateral direction, inter-electrode distances can vary between the two dimensions. However, this will prevent the use of bi-dimensional spatial filters. - If the data are to be analyzed in monopolar montage, the detection volume is not influenced by the inter-electrode distance. If spatial filters are to be applied, a balance between higher spatial resolution (smaller inter-electrode distance) and larger detection volume (larger inter-electrode distance) should be considered. - Inter-electrode distances >10mm may result in spatial aliasing, which does not allow the interpolation of the spatial potential distribution. For very thin skin and subcutaneous layers (<1.2mm) the IED should be limited to 3-5 mm. - Too small inter-electrode distance between electrodes may cause a short circuit between the electrodes because of sweat or gel/paste leakage.	- An accurate estimation of average muscle fiber conduction velocity depends on the presence of action potentials from the same muscle fibers on at least 2 sEMG channels along the muscle fiber direction. This can be verified by calculating the cross-correlation coefficient between the sEMG signals used to estimate conduction velocity; correlation coefficients of 0.75 or higher are usually considered necessary to estimate conduction velocity. It should be noted that the presence of non-propagating components will also result in large cross-correlation between sEMG signals, while biasing conduction velocity estimates towards high value. Larger numbers of electrodes result in a larger number of channels, improving the estimation of average muscle fiber conduction velocity. - Larger inter-electrode distances will result in lower precision in the estimation of the location of the innervation zone and of the muscle-tendon regions. - For applications where spatial interpolation is needed (muscle fiber orientation, location of muscle fibers innervated by a single motoneuron), inter-electrode distances >10mm may result in spatial aliasing, which degrades the interpolation of the spatial distribution. For very thin skin and subcutaneous layers (<1.2mm) the IED should be limited to 3-5 mm.	- When large inter-electrode distances (>5mm for small muscles, e.g., hand and face; >10mm for larger muscles) are used, each single motor unit action potential is only detected by few channels. This may cause the spatial distribution of the action potential to appear similar between different motor units, hindering the accurate identification of single motor units.
Recommended use	- Bi-dimensional adhesive arrays are generally recommended compared to linear arrays. - In muscles with pennate architecture in depth direction, adhesive linear arrays can be considered when a single dimension is of interest (e.g., if only the cranio-caudal or the medio-lateral EMG amplitude distribution are of interest). In muscles with fibers parallel to the skin, adhesive linear arrays can only be considered if their electrodes are placed on the same muscle fiber region across the muscle of	- Bi-dimensional adhesive arrays are generally recommended compared to linear arrays. - A dry linear repositionable array is instead recommended when searching for the innervation zone and/or the approximate fiber orientation for subsequent placement of conventional bipolar or other sEMG system. - To estimate average muscle fiber conduction velocity, linear or bi-dimensional arrays with more than 4 electrodes along the muscle fiber direction (resulting in	- Bi-dimensional adhesive arrays are generally recommended for the identification of single motor units. - For the identification of single motor units, small inter-electrode distances (≤ 5mm for small muscles, e.g., hand and face; ≤ 10mm for larger muscles) should be used. - A larger number of channels may result in a better discrimination of action potentials.

	interest (e.g., above the innervation zone for monopolar recordings). - Dry electrodes should be considered when the use of adhesive electrodes is not ideal or impossible (e.g., anal probe). - Dry electrodes should be considered when short setup and data collection time are necessary (e.g., clinical applications, studies on children). - If the data are to be analyzed in monopolar montage, smaller inter-electrode distances (better spatial resolution) are generally recommended (compatibly with the hardware available and the experimental question). - If the data are to be analyzed after spatial filtering, the inter-electrode distance should be chosen to balance spatial resolution (improved by smaller inter-electrode distances), detection volume (improved by larger inter-electrode distances) and array size. - Inter-electrode distance should be small enough to prevent spatial aliasing and allow interpolation (values between 2.5 mm and 10 mm are acceptable).	the minimum of 2 double differential signals) are recommended. - For the identification of the location of the innervation zone and of the muscle-tendon region, smaller inter-electrode distances (5 mm or less in medium and large muscles; 2.5 mm or less for small muscles) are recommended to increase the spatial resolution of the measure, in particular for very superficial muscles.	
Non-recommended use	- Inter-electrode distances > 10mm should not be used if spatial interpolation needs to be applied.	- Inter-electrode distances > 10mm should not be used if spatial interpolation needs to be applied.	- Linear arrays, or bi-dimensional arrays with large inter-electrode distances (>5mm for small muscles, e.g., hand and face; >10mm for larger muscles), should not be used for motor unit decomposition because they may yield a smaller number of motor units compared to bi-dimensional arrays with small inter-electrode distances. However, further research is necessary to assess the effect of inter-electrode distance on the number of motor units obtained by decomposing HDsEMG signals.
To report	- Electrode type and size, number of electrodes, spatial organization, inter-electrode distance.	- Electrode type and size, number of electrodes, spatial organization, inter-electrode distance.	- Electrode type and size, number of electrodes, spatial organization, inter-electrode distance.
c) Electrode location and orientation			

General considerations	- In muscles with fibers parallel to the skin: electrodes placed along the muscle fiber direction will detect the same action potential propagating along the muscle fiber. Because of this redundancy, regional variations in amplitude along the muscle fiber direction are generally not associated with regional activation. Instead, regional activation may be observed as variations in amplitude recorded by electrodes placed over different muscle fibers (i.e.: transverse to the muscle fiber direction). If the electrode array is placed on a skin region over several different muscles (e.g., the forearm extensors), activation of different muscle may be observed along both dimensions. - In muscles with pennate architecture in depth direction: each electrode will be placed on the location where a different group of fiber inserts on the superficial aponeurosis. For this reason, regional activation can be observed as changes in amplitude distributions in both dimensions and propagation is difficult to observe.	- In muscles with fibers parallel to the skin: location and orientation of the HDsEMG electrodes highly depend on the feature that needs to be extracted. Specific applications are detailed in the "recommended use" section. - In muscles with pennate architecture in depth direction, the following fiber membrane properties cannot be extracted: average muscle fiber conduction velocity, location of the main innervation zone, location of muscle-tendon regions, fiber orientation on the plane of the skin, length of muscle fibers.	- There is no clear recommendation on which HDsEMG electrode orientation and location yields the largest number of accurately identified single motor units. - In muscles with fibers parallel to the skin: as differences in the spatial action potential distribution appears to be a critical factor in the identification of single motor units, it is possible that HDsEMG array location and orientations that provide the most diverse spatial action potential distribution between motor units are to be preferred. These may include collecting HDsEMG from: muscle regions with more pennate architecture in depth direction (e.g., proximal region of the tibialis anterior, compared to the distal region); above the innervation zone compared to along the muscle fiber; electrodes-oriented transverse to the muscle fiber orientation. This needs to be confirmed in experimental studies.
Cautions	- If the electrodes on the edge of the HDsEMG array are placed outside of the muscle boundaries, there is an increased risk of crosstalk from neighboring muscles. On the other hand, if an array covers only a portion of a muscle there is truncation of the signal at the edge. This may cause problems in some processing (e.g., spectrum in space). Similarly, regional activation identified from a muscle with mixed architecture will reveal large differences in amplitude between regions (generally larger on the region with fibers parallel to the skin, and smaller on the region with pennate architecture in depth direction). - It should be considered that crosstalk can be present even if the electrodes are well within the muscle boundaries. Furthermore, crosstalk is more likely to be present if the electrodes are close to the	- In muscles with fibers parallel to the skin: changes in peak amplitude over consecutive channels located between the innervation zone and the tendon insertion may indicate misalignment between the surface array and the orientation to the muscle fibers, or changes in the thickness or composition of the tissues between the muscle and the HDsEMG electrodes. This can affect the estimation of conduction velocity. - Some muscles (e.g., sartorius) may have several innervation zones along their muscle length. With current technology, conduction velocity may be estimated from the multiunit signal only if there is unidirectional propagation. - Some muscles (e.g., facial muscles, external anal sphincter) may have curved fibers and innervation zones located far from the middle of the muscle fiber.	- Large variations in the number of motor units accurately identified from different muscles have been observed (Del Vecchio et al., 2020). Depending on the participant and on the task, in some muscles (tibialis anterior, medial gastrocnemius) it is possible to extract tens of motor units, in others (biceps brachii, lateral gastrocnemius, vastii) less than ten. It is also possible that, in some participants, no motor units can be accurately identified. Thickness of subcutaneous tissues and muscle architecture, such as the similarity of action potentials along the muscle fibers, may play a role. Further studies are needed to understand the reason of the between-muscle and between-participant differences in the number of accurately identified motor units.

	boundaries and when there are larger amounts of subcutaneous adipose tissue. - When spatially filtered sEMG signals are considered, misalignment between the muscle fiber orientation and the electrodes results in lower sEMG amplitude. It should be noted that, if a muscle has a fan-shaped architecture (e.g., vastus medialis, pectoralis major) and the electrode array has parallel columns of electrodes, it will be impossible to align all the electrode columns with the muscle fiber orientation in all the muscle regions. This may be erroneously interpreted as regional activation. - In muscles with fibers parallel to the skin, the spatial distribution of muscle activation is different between single differential signals (low amplitude above the innervation zone, high amplitude along the muscle fiber direction) and monopolar montages, double differential, and Laplacian signals: (high-amplitude above the innervation zone). If the array is applied to cover only a region of the muscle, whether the innervation zone should be included in the recording area or not depends on the electrode montage and the purpose of the measurement. This does not apply to muscles with a pennate architecture in depth direction. - Local differences in the underlying tissue composition, geometry and conductivity between the muscle fibers and the electrodes could result in differences in signal amplitude which could be misinterpreted as differences in regional activation.		
Recommended use	- HDsEMG electrodes should be placed in a position and orientation that allows sampling of electrical activity from the different muscle regions of interest. - Muscle boundaries and aponeuroses should be identified using ultrasound or anatomical references (if possible), and electrodes outside the area of interest should be excluded from processing.	- To identify the position of the innervation zone in muscles with fibers parallel to the skin, it is recommended to orient the HDsEMG electrodes along the muscle fiber direction. In most muscles, the innervation zone can be located on the skin near the middle of the muscle belly. - To identify the position of the muscle-tendon region in muscles with fibers parallel to the skin, it is	- To identify motor units representative of the whole muscle, as opposed to a single muscle region, it is recommended to position the array of surface electrodes in a position and orientation so that the electrodes span as much as possible of the muscle of interest. - If single motor unit firings will be used to obtain the action potential spatial distribution

	- In muscles with fibers parallel to the skin, regional activation cannot be observed along the muscle fiber direction; hence the array should have a sufficient number of electrodes in the transverse direction. - In muscles with fibers parallel to the skin, the location of the innervation zone should be identified before placing the HDsEMG arrays in order to place the array in the desired position. - In muscles with fibers parallel to the skin, the electrode array should be placed over the innervation zones of the regions of interest if the data are analyzed in monopolar montage (because sEMG amplitude is larger over the innervation zone compared to along the muscle fiber). - In muscles with fibers parallel to the skin, the electrode array should be placed proximal or distal to the innervation zones of the regions of interest if the data are analyzed in single differential montage. - In muscles with fibers parallel to the skin, the possible excursion of the innervation zone due to changes in joint angle or to muscle force production should be known and accounted for when placing the electrode array; ensure that it is under the array (monopolar montage) or proximal/distal to the array (single differential montage) throughout the task. The user should be aware of the fact that the signal amplitude may change because of movement of the muscle under the skin. - In muscles with pennate architecture in depth direction, the HDsEMG must be placed over the target muscle region, regardless of the electrode montage. - In muscles with mixed architecture (e.g., medial gastrocnemius, which has a pennate architecture in depth direction in the proximal region and fibers parallel to the skin the distal region), regional differences in anatomy should be identified and the HDsEMG array should be placed accordingly.	recommended to orient the HDsEMG electrodes along the muscle fiber direction. The HDsEMG electrodes should be centered over the muscle-tendon region, identified using ultrasound or anatomical references. - To identify the approximate muscle fiber orientation with a dry repositionable array in muscles with fibers parallel to the skin, it is recommended to orient the array along the expected fiber orientation based on the muscle anatomy. The array should be centered between the innervation zone and the muscle-tendon region to be able to observe propagation in as many channels as possible to determine the appropriate orientation. - To identify the muscle fiber orientation of motor units located in different muscle regions in muscles with fibers parallel to the skin, it is recommended to use a bi-dimensional HDsEMG array placed over the muscle region of interest, comprising the innervation zone and the muscle-tendon region. - To estimate the location of muscle units (muscle fibers of a single motor unit) both in muscles with fibers parallel to the skin and in muscles with pennate architecture in depth direction, it is recommended to use a bi-dimensional HDsEMG placed over the muscle region of interest, or a linear array placed transverse to the muscle fiber orientation. Linear arrays can be used in muscles with pennate architecture in depth direction, but the location of muscle units will be determined in one dimension only.	(by triggered-averaging surface sEMG signals; see Data Analysis) to investigate muscle fiber properties, the HDsEMG array position and orientation should be decided according to the indication of the relevant application. For instance, if the aim is to measure average muscle fiber conduction velocity of individual motor units, the HDsEMG electrodes should be oriented along the muscle fiber and have the largest possible number of channels proximal or distal to the innervation zone.

Non-recommended use	- In muscles with fibers parallel to the skin, if non-isometric or strong isometric contractions are performed, the use of a linear array placed transverse to the fiber direction is not recommended, as changes in sEMG spatial amplitude distribution due to shifts of the innervation zone under/proximal or distal to the electrode and changes in muscle shape can be erroneously interpreted as changes in regional activation.	- The location of the innervation zone, muscle fiber conduction velocity, muscle-tendon region, muscle fiber length and orientation cannot be identified from a linear array placed transverse to the muscle fiber direction.	- When the firing patterns of the identified motor units are intended to be as representative as possible of the whole muscle, the array should not cover only a limited region of the muscle. When the aim is to obtain firing patterns as representative as possible of the whole muscle, motor units should not be identified from an array that covers only a relatively small region of the muscle.
To report	- How the anatomical references were used to determine location and orientation of the array (e.g., ultrasound, known anatomical references). - Location and orientation of the array with respect to the anatomical references (e.g., expected fiber orientation).	- How the anatomical references were used to determine location and orientation of the array (e.g., ultrasound, known anatomical references). - Location and orientation of the array with respect to the anatomical references (e.g., expected fiber orientation).	- How the anatomical references were used to determine location and orientation of the array (e.g., ultrasound, known anatomical references). - Location and orientation of the array with respect to the anatomical references (e.g., expected fiber orientation).
d) Data analysis			
General considerations	- Regional activation is generally evaluated based on the intensity of the sEMG signal (e.g., RMS value) recorded by electrodes placed over different muscle regions. Various methods exist to define the location and extent of the active area(s) of interest. - It should be noted that most of the information provided here also applies to changes in spatial distributions of mean/median frequency values during fatiguing contractions.	- When estimated from the multiunit signal, muscle fiber properties estimates represent an average value of all the motor units in the detection volume (although motor units with larger surface potentials will have a larger weight on the average). If paired with single motor unit decomposition, it is possible to obtain these estimates for individual motor units. It is not possible to use surface array electrodes to calculate single muscle fiber conduction velocity.	- Single motor unit identification algorithms use information on the spatial distribution of action potentials to discriminate firings belonging to different motor units. Superimposition of the motor unit action potential of different motor units is resolved with iterative processes.
Implementation	- If the HDsEMG signal is stationary (meaning that its statistical properties do not vary over time, e.g., isometric contraction at a constant force level and for limited time), the intensity of the muscle activation is generally calculated as the Root Mean Square or the Average Rectified Value over a predefined time window and for each channel. - If the HDsEMG signal is non-stationary (e.g., isometric contraction at a varying force level, non-isometric contractions, functional tasks), the intensity of the muscle activation is generally calculated as the Root Mean Square or the Average Rectified Value	- Muscle innervation zones are usually identified visually (inversion of the polarity and start of the propagation of action potentials), as a change of direction/sign of muscle fiber conduction velocity, as a drop of sEMG amplitude in 1-2 channels in single differential montages, or as a peak of sEMG amplitude in monopolar, double differential or Laplacian montages. - The muscle-tendon region is usually identified by observing the channel in which the motor unit action potential propagation stops (small/no delay between consecutive channels, single differential montage).	- Single motor unit identification is usually performed using specialized software, typically based on blind source separations techniques (although more traditional spike detection and sorting remains in use as well). Users provide minimal input on the motor unit identification process, the main input being the number of iterations the algorithm must perform. Larger number of iterations provide more accurately identified motor units. - A critical, user-dependent step in the accurate identification of motor units is the

	over a predefined time period. However, compared to stationary signals, shorter epochs may be used to be able to describe regional changes in muscle activation as a function of time. In any case, epochs should be 125 ms or longer to limit variability of the estimate. - If a higher temporal resolution is needed, for instance to perform cross-correlation analysis between regional activation observed with HDsEMG and other physiological signals, or to apply factorization algorithms, it is common practice to calculate the envelope of individual channels by low-pass filtering the rectified (or squared) sEMG signal collected by each channel or by calculating RMS/ARV with a sliding window. - If muscle activation is triggered by an external event, such as a perturbation or an evoked potential, responses are generally described using peak-to-peak amplitude, or by calculating Root Mean Square or the Average Rectified Value over the time window where a response can be observed.	- The approximate muscle fiber orientation is generally estimated by visually assessing the sEMG signals collected during low-force contractions with the array oriented at different angles. Action potentials appearing with similar amplitude in consecutive channels, and with delay compatible with physiological conduction velocity values (usually 2-3 ms per channel for inter-electrode distance = 10 mm and conduction velocity = 3-5 m/s), indicate alignment between the array and the approximate fiber orientation. - Average muscle fiber conduction velocity is generally calculated from electrodes placed along the approximate fiber orientation, or with techniques that combine information from channels in different locations along the muscle fiber direction. - The muscle fiber orientation of individual motor units is usually identified from the average spatial distribution of the single motor unit action potential, which is obtained by spike-triggered averaging the sEMG signal in each HDsEMG electrode (see Data analysis – Single motor unit activity). Tracking of the spatial characteristics of the action potential propagation is performed by identifying the peak of the distribution at each time frame between the action potential generation and extinction. Signals are usually analyzed in monopolar montage, after spatial interpolation. Only the polarity showing action potential propagation is generally tracked, whereas the opposite polarity representing action potential generation and end-of-fiber effect is usually not considered. - In muscles with fibers parallel to the skin, the location of the muscle fibers innervated by a single motoneuron is generally calculated from the sEMG amplitude distribution obtained after spike-triggered averaging (see Data analysis – Single motor unit activity). In monopolar, double differential and Laplacian montages, this distribution usually has a single peak that corresponds to the location of the motor unit	estimation of errors in the identification of motor unit firings. Accurate decomposition of multiunit signals into single motor unit firing trains is usually assessed visually or using metrics such as the pulse to noise ratio. Single motor unit firing trains showing improbable firing patterns, such as unexpectedly high or low mean firing rate (e.g., >50 pulses/s in a low-force isometric contraction) or large coefficient of variation (>0.3), are reviewed manually and often excluded and removed from the pool of identified motor units. - Single motor unit firing trains showing transient episodes of non-physiological firing patterns are usually manually corrected. Some motor unit decomposition softwares provide visualization of the instantaneous pulse-to-noise ratio, which allows the identification and correction of missed and erroneously identified firings.

		innervation zone. In single differential recordings, the spatial distribution will have higher amplitude values along the single motor unit fibers, and low values above the innervation zone. In muscles with pennate architecture in depth direction, the spatial distribution will have high amplitude values on the electrodes placed over the superficial region of the fibers belonging to the motor unit under exam.	
Data extraction	- Arrays with poor or unstable electrode-skin contact may be identified as channels with large power line interference, noise, or artifacts. If these channels are few (<10%) and isolated, they may be removed and sometimes replaced by the sample-by-sample average of the neighboring channels. If these channels are many or clustered in groups, the recording should be discarded and repeated. HDsEMG signals should be checked in real-time during data collection to identify whether the signal quality is acceptable or not, and if the task should be repeated. - Changes in the intensity of sEMG distribution are usually described as spatial changes in the RMS or ARV amplitude over time. - Changes in distribution of sEMG activation are usually described by calculating the centroid (or center of mass, where the mass is the signal amplitude) of the spatial sEMG amplitude distribution. The centroid consists of a spatial coordinate (or two in the case of bi-dimensional arrays). If the regional activation shifts during a contraction or between tasks, the centroid will shift towards the region of the HDsEMG channels with higher amplitude. It should be noted that, unless the less active region has amplitude values close to 0, the centroid may be located far from the region with largest amplitude. - The definition of a region of activity is sometimes used to extract intensity, location, and extent of the active muscle area. This is commonly done by selecting channels with values higher than a pre-	- The location of the innervation zone is usually described as distance from anatomical references (in cm) or as the number of the channels showing smaller amplitudes in single differential montage. The precision of the measure can exceed the inter-electrode distance if interpolation or methods based on image processing are used. - The location of the muscle-tendon region is usually measured as the distance from an anatomical reference (in cm) or as the number of the channel at which action potential propagation stops. - The approximate muscle fiber orientation measured with a dry array can be calculated as the angle between the orientation of the electrode array (aligned with the muscle fiber direction) and an anatomical reference line. - In muscles with fibers parallel to the skin, the muscle fiber length can be extracted visually (by identifying the muscle-tendon region at the origin and insertion of the muscle, assuming that the muscle fibers run along the whole muscle length and are aligned with a long enough electrode array), from recordings spike-triggered averaged from motor units (see Data Analysis – Single Motor Unit Activity; by following the action potential propagation from generation to extinction), or by combining information on timing of action potential generation, end-of-fiber effect, and average muscle fiber conduction velocity. - Average muscle fiber conduction velocity is usually calculated as the distance between detection points divided by the time shifts between the sEMG signals	- Most of the temporal information on the instants of firing provided by classical, intramuscular recordings can also be obtained by decomposition of HDsEMG recordings. Common indices extracted are firing rate, coefficient of variation of interspike interval, recruitment/de-recruitment threshold. - The sum of the trains of discharge instants of the identified motor units is often referred to as the cumulative spike trains. The cumulative spike train is an estimate of the neural drive to the muscle and has a strong association with force. The strength of the association depends on the number of identified motor units. - Muscle fiber properties of individual motor units can be investigated by obtaining the sEMG representation of the average action potential of individual motor units. This can be extracted by spike-triggered averaging, which consists of averaging sEMG signals in a fixed time window (e.g., 60 ms) centered on each firing of the selected motor unit. When averaging, the action potential of motor units other than the selected one will not be synchronized and will cancel each other. Instead, the shape of the target motor unit will consistently appear in the center of each time window and will then be maintained in the average signal. When repeated for each HDsEMG channel, this process will reveal the

	defined threshold. In the absence of muscle-specific thresholds from in-vivo studies, simulation studies indicate 70% of the peak amplitude of the sEMG distribution as a threshold to identify the location of active motor units positioned under the array. Once a region of interest is defined: i) the intensity of sEMG activation can be calculated as the average RMS amplitude of the channels in the region of activity; ii) the location of the activation can be described as the centroid of the channels in the region of activity; and iii) the extent of the active muscle area can be described as the number of channels in the region of activity. In general, the location of activation estimated after definition of the region of activity will be located closer to the peak of the sEMG amplitude distribution than the centroid calculated on all channels of the array. - Changes in distribution of sEMG activation are sometimes described by calculating the coordinates of the peak of the sEMG amplitude distribution. However, this method should only be used when the sEMG amplitude distribution clearly shows a single peak. In addition, the location of the peak is critically affected by the presence of channels with strong noise, power line interference, or artifacts. - Regional activation has also been recently described using factorization algorithms such as principal component analysis and non-negative matrix factorization on envelopes calculated from individual HDsEMG channels. This processing can be applied to determine the common spatial features of HDsEMG recordings across individuals, and how the temporal activation of these components varies in time.	recorded at these points (different channels of the array aligned along the fiber direction). Average muscle fiber conduction velocity can be estimated using multiple channels along the same array column and along nearby columns. The cross-correlation coefficient between channels used to calculate average muscle fiber conduction velocity is usually reported as an index of similarity between potential sEMG shapes in different channels. The time shift is usually estimated in the frequency domain to avoid the limit in temporal resolution imposed by the sampling period. - The muscle fiber orientation of individual motor units it is usually displayed visually either in a figure or calculated as the angle between the linear fit of the locations of the action potential peaks during propagation and an anatomical reference. - Two parameters associated to the distribution of fibers innervated by a single motoneuron can be extracted using HDsEMG: i) the location of the spatial distribution of the motor unit action potential on the skin, which is associated to the average position of the muscle fibers of a motor unit projected on the skin plane; ii) the spread of the spatial distribution of the motor unit action potential, which is associated to the motor unit territory (the area within a muscle physiological cross-sectional area in which the muscle fibers of a single motor unit are distributed). The motor unit position is usually reported as the coordinates of the peak or of the centroid of the region of interest. In muscles with fibers parallel to the skin, the spread of the spatial distribution is calculated after determination of each motor unit's fiber orientation. One possible method consists of fitting a Gaussian distribution to the spatial amplitude distribution of the surface action potential, transverse to the fiber orientation. The standard deviation of this distribution is reported as a measure of spread of the spatial distribution of the motor unit action potential. In muscles with pennate architecture in depth direction, both the proximal-distal	action potential distribution on the skin for each motor unit.

		and the medial-lateral direction are considered to be transverse to the fiber orientation.	
Cautions	- The presence of "bad" channels with strong noise, power line interference, or artifacts can drastically influence the estimation of regional activation from HDsEMG recordings. - The presence of crosstalk from neighboring muscles can influence the estimation of regional activation. - The threshold of 70% of the peak amplitude used to identify of regions of activity is based on results from simulations. Experimental studies are necessary to validate these findings in-vivo and for different muscles. - During fatiguing tasks changes in sEMG amplitude distribution may be due to factors other than region-specific changes in neural drive to the muscle (e.g., local changes in muscle fiber conduction velocity). - Variations in volume conductor properties (such as tissue inhomogeneities, geometrical and electrical properties) could also influence estimates of regional activation.	- Only superficial muscles with fibers parallel to the skin are suitable for average muscle fiber conduction velocity estimation. - The result of the average muscle fiber conduction velocity estimate is a weighted average of the muscle fiber conduction velocities of the motor units in the detection volume. As the estimate is based on the lag of the peak of the cross-correlation between multiunit signals, motor units with larger action potentials have greater weight in determining this lag than smaller or deeper motor units. - It should be considered that the average muscle fiber conduction velocity is overestimated when the distance of the muscle fibers from the skin surface increases, or if the HDsEMG electrodes are misaligned with respect to the fiber orientation. - It should be considered that tissue inhomogeneities can cause errors in the measured average muscle fiber conduction velocity. - Muscles may have multiple innervation zones. HDsEMG only allows the identification of the location of the innervation zone of superficial motor units.	- Occasional motor unit firings with interspike intervals shorter than expected (e.g., doublets) may be erroneously classified as outliers and removed from the analysis. In intramuscular signals, visual analysis of the shape of the action potential can assist in determining whether the two firings belong to the same motor unit or not. This is possible, although less direct, also with HDsEMG recordings. In this case, although direct visual identification of potentials belonging to the same motor unit is very difficult, firings can be checked visually after repeated (iterative) application of separation filters (for details, see (Del Vecchio et al., 2020)).
Recommended use	- When calculating Average Rectified Value and Root Mean Square, it is recommended to use time epochs not shorter than 125ms (to limit variability of the estimate) and not longer than 2 s (to limit the effect of non-stationarity of the signal). - Ensure that the location of the electrode and the anatomy of the muscle underneath is known and considered in the interpretation of the results.	- For the estimation of average muscle fiber conduction velocity, the selection of channels with cross-correlation coefficient >0.75 is a necessary but not sufficient condition. Visual assessment is recommended. The presence of non-propagating potentials (common mode signal, end-of-fiber effects) cause overestimates of the conduction velocity value despite high correlation coefficients. - There is no recommendation about which channels should be used for calculating the time shift (adjacent or not). However, it should be considered that larger distances between channels increase the risk of tendon, endplate, or inhomogeneity effects, while averaging more average muscle fiber conduction velocity values	- It is recommended to visually check the spike trains of each identified motor unit, manually editing the firing times when possible or excluding the motor unit when necessary.

		resulting from adjacent channels with small inter-electrode distances reduces this risk. - When using bi-dimensional arrays, algorithms that account for misalignment between electrodes and fiber orientation should be considered.	
Non-recommended use	- Time epochs shorter than 125 ms are not recommended to calculate amplitude or frequency indicators.	- Average muscle fiber conduction velocity should not be calculated when the electrodes are not aligned with the muscle fibers, or using electrodes close to the innervation zone or to the muscle-tendon region.	- Motor unit firing times extracted from HDsEMG using decomposition algorithms should not be analyzed without ensuring that the results of the automatic identification are within physiologically plausible range. - Decomposition methods validated only for isometric contractions should not be used to identify motor units from HDsEMG signals collected during dynamic tasks.
To report	- Indicate the number of channels excluded from the analysis or replaced by interpolation. - Indicate the time epoch used for estimation of amplitude, or spectral parameters or CV. - Describe the processing used to obtain the spatial sEMG amplitude distribution. - Describe if a region of activity was determined, and how. - Describe how the centroid was calculated.	- Algorithm used for estimation of conduction velocity; number and location of channels used. - Cross-correlation coefficients should be reported when reporting average muscle fiber conduction velocity values.	- Method for decomposing HDsEMG signals. - Number of motor units extracted, number of motor units analyzed, general firing characteristics (e.g., number of firings, firing rate, coefficient of variation). - If spike-triggered averaging is performed, indicate the number of motor unit firings used to compute the analysis. - Metric of the quality of the decomposition (for example pulse to noise ratio).
e) Interpretation			
General considerations	- Consistent changes or differences in sEMG amplitude spatial distribution measured with HDsEMG can be interpreted as changes in activation of regions within a muscle or muscle group. However, as changes in sEMG amplitude depend on both changes in neural drive (motor unit recruitment/de-recruitment, motor unit firing rate) and muscle fiber properties (e.g., muscle architecture, average muscle fiber conduction velocity), regional activation must be interpreted carefully.	- Average muscle fiber conduction velocity is associated with motor unit size (larger motor units have larger fiber diameters and higher conduction velocity). Changes in average muscle fiber conduction velocity during constant-force isometric contraction indicate changes in the ionic concentrations and ionic channel dynamics across the sarcolemma. - Studies on the identification of innervation zone and muscle-tendon region location, fiber orientation and length, and motor unit location would benefit from validation with other gold-standard techniques (e.g., ultrasound, intramuscular EMG, imaging, and anatomical dissection studies).	- The extraction of single motor unit firing patterns from HDsEMG has been shown to be valid when compared to gold-standard intramuscular electromyography in isometric contractions.

		- Changes in the location of innervation zones indicate changes of muscle length in non-isometric contractions.	
Cautions	- Within-muscle differences in sEMG amplitude spatial distribution may be due to factors not associated with regional activation, such as different type or thickness of tissues interposed between the recording system and the muscle, differences in pennation angle, misalignment of the electrode array with respect to the muscle fiber direction (when spatial filters are applied). - Between-subject differences in sEMG amplitude spatial distribution may be due to factors not associated with regional activation, such as differences in tissues interposed between the muscle of interest and the HDsEMG system and differences in muscle architecture. - Within-subject changes in sEMG amplitude spatial distribution may be due to factors not associated with regional activation, such as changes in average muscle fiber conduction velocity during fatiguing contractions (slowing of the action potential propagation increases the amplitude of the surface sEMG, despite constant neural drive) and changes in muscle architecture in non-isometric or high-force contractions (e.g., shift of the innervation zone, shift of the muscle fiber). -In non-isometric contractions, or in contractions at different joint angles, the muscles may move under the electrode array and the region of activity may shift.	- Estimates of average muscle fiber conduction velocity, innervation zone location, muscle-tendon region, approximate fiber orientation and fiber length represent an average value for the motor units in the detection volume, with larger weights for motor units contributing larger surface action potentials (i.e.: more superficial, larger, or better aligned with the electrodes). Characteristics or firing patterns of individual motor units within the sample may differ. For this reason, estimates from one muscle region should not be assumed to be representative of the whole muscle, as there may be regional variations in conduction velocity, muscle fiber orientation, etc. - In the estimation of the spread of the spatial distribution of the motor unit action potential, the standard deviation of the Gaussian fitting is associated to the location in space of most (not necessarily all) of the muscle fibers innervated by a single motoneuron. In muscles with fibers parallel to the skin, this measure is also affected by other factors such as motor unit depth and should undergo further assessment. - Staining techniques suggest that the innervation zones are not as discreet as electrophysiological recordings suggest (Mu and Sanders, 2010). It should be considered that only the innervation zone of superficial motor units, where action potential propagation can be clearly observed, can be identified using HDsEMG. HDsEMG provides an indication of distribution of innervation zones, which is not necessarily comparable to estimates with staining techniques.	- Changes in the number of motor units identified are not necessarily associated with the number of motor units recruited/derecruited in the muscle. It is possible to observe fewer accurately identified motor units at higher compared to lower contraction levels. This is associated with difficulties in identifying single motor unit firings due to increased superimposition of motor unit action potentials, as opposed to physiological changes in the number of single motor units recruited. - Motor units identified from HDsEMG recordings are likely to be located superficially in the muscle. This may be especially relevant when motor units are identified from single differential signals with small inter-electrode distance (or other highly selective spatial filters). Firing patterns are unlikely to be representative of deeper motor units.
To report	- Steps taken to limit the effects of factors not associated with neural drive on the estimation of regional activation.	- Assumptions made during data analysis, if any. - Comparison of results with those obtained with techniques other than HDsEMG (e.g., imaging or dissection for muscle fiber orientation), when available.	- Acknowledge that the results are valid for a population of superficial motor units, which may not be representative of the entire muscle.

APPENDIX:

Appendix 1. Delphi rating scores. Each cell provides median score and (in parenthesis) IQR in first row, then % appropriate (scores 7–9) followed by inappropriate (scores 1–3) in second row.

HDsEMG matrix items	Round	Rating scores – Median (IQR); % appropriate (n), % inappropriate (n)		
Definition	1	8 (1.5); 84.6% (11), 0% (0)		
General considerations	1	7 (1); 92.3% (12), 0% (0)		
Applications of HDsEMG		***Regional activation***	***Muscle fiber properties***	***Single motor unit activity***
Definitions	1	8 (1) 100 (13), 0 (0)	8 (1.5) 76.9 (10), 0 (0)	8 (1) 92.3 (11), 0 (0)
Examples of applications for the assessment of neuromuscular function in health and pathology	1	8 (0.5) 94.6 (11), 0 (0)	8 (1) 84.6 (11), 7.7 (1)	8 (0) 100 (13), 0 (0)
Tasks or experimental condition	1	8 (2) 84.6 (11), 0 (0)	8 (2) 100 (13), 0 (0)	8 (2) 100 (13), 0 (0)
Electrode montage		***Regional activation***	***Muscle fiber properties***	***Single motor unit activity***
Description	1	8 (**3.5**) **69.2** (9), 7.7 (1)	8 (2) 84.6 (11), 7.7 (1)	8 (2) 92.3 (12), 0 (0)
	2	8 (1) 92.9 (13), 0 (0)	8 (1.3) 85.7 (12), 0 (0)	8.5 (1) 92.9 (13), 0 (0)
Pros	1	8 (1.5) 10 (13), 0 (0)	8 (1.5) 92.3 (12), 0 (0)	7 **(3)** **61.5 (8), 15.4 (2)**
	2	8 (1) 92.9 (13), 0 (0)	8 (1) 92.9 (13), 0 (0)	8 (1) 100 (14), 0 (0)
Cons	1	8 (0.5) 92.3 (12), 0 (0)	8 (1) 100 (13), 0 (0)	8 **(2.5)** **69.2 (9), 15.4 (2)**
	2	8 (1) 85.7 (12), 0 (0)	8 (1) 92.9 (13), 0 (0)	8 (2.3) 78.6 (11), 0 (0)
Recommended use	1	8 (1) 92.3 (12), 0 (0)	8 (1) 92.3 (12), 0 (0)	9 (1) 92.3 (12), 0 (0)
Non-recommended use	1	8 (1.5) 84.6 (11), 0 (0)	8 (1.5) 92.3 (12), 0 (0)	8 **(2.5)** **69.2 (9), 15.4 (2)**
	2	8 (1) 100 (14), 0 (0)	8 (1) 100 (14), 0 (0)	8 (1) 85.7 (12), 0 (0)
To report	1	8 (1) 92.3 (12), 0 (0)	8 (1) 92.3 (12), 0 (0)	8 (1) 92.3 (12), 0 (0)
Electrode type and configuration		***Regional activation***	***Muscle fiber properties***	***Single motor unit activity***
General considerations	1	8 (1) 92.3 (12), 0 (0)	8 (1) 92.3 (12), 0 (0)	8 (1) 100 (13), 0 (0)
Cautions	1	9 (1) 100 (13), 0 (0)	8 (1) 92.3 (12), 0 (0)	8 (1) 84.6 (11), 0 (0)
Recommended use	1	8 (2) 76.9 (10), 7.7 (1)	8 (1) 92.3 (12), 0 (0)	8 (1.5) 92.3 (12), 0 (0)
Non-recommended use	1	8 (1) 100 (13). 0 (0)	8 (1) 100 (13), 0 (0)	8 **(4.5)** **61.5 (8), 23.1 (3)**
	2	9 (1) 100 (14), 0 (0)	9 (1) 100 (14), 0 (0)	8 (2) 92.9 (13), 0 (0)
To report	1	8 (1) 92.3 (12), 0 (0)	8 (1) 92.3 (12), 0 (0)	8 (1) 92.3 (12), 0 (0)
Electrode location and orientation		***Regional activation***	***Muscle fiber properties***	***Single motor unit activity***
General considerations	1	8 (1) 84.6 (11), 7.7 (1)	7 **(2.5)** **69.2** (9), 7.7 (1)	8 (2) 92.3 (12), 7.7 (1)

	2	9 (1.5) 78.6 (11), 0 (0)	8 (1) 85.7 (12), 0 (0)	8.5 (1) 92.9 (13), 0 (0)
Cautions	1	8 (1.5) 84.6 (11), 7.7 (1)	8 (1) 84.6 (11), 7.7 (1)	**6 (5)** **46.2 (6), 23.1 (3)**
	2	8 (1.3) 100 (14), 0 (0)	8.5 (1) 100 (14), 0 (0)	8 (1) 100 (14), 0 (0)
Recommended use	1	7 (2) **69.2** (9), 7.7 (1)	8 (0.5) 92.3 (12), 7.7% (1)	8 (1) 100 (13), 0 (0)
	2	8 (1) 100 (14), 0 (0)	8 (1.3) 85.7 (12), 0 (0)	8 (1) 92.9 (13), 0 (0)
Non-recommended use	1	8 (1) 92.3 (12), 7.7 (1)	8 **(3)** 76.9 (10), 7.7 (1)	8 **(3)** 76.9 (10), 7.7 (1)
	2	9 (1) 92.9 (13), 0 (0)	8 (1) 100 (14), 0 (0)	8 (2) 92.9 (13), 0 (0)
To report	1	8 (1) 92.3 (12), 0 (0)	8 (1) 92.3 (12), 0 (0)	8 (1) 92.3 (12), 0 (0)
Data analysis		***Regional activation***	***Muscle fiber properties***	***Single motor unit activity***
General considerations	1	8 (1.5) 84.6 (11), 0 (0)	8 (1) 100 (13), 0 (0)	8 (1) 92.3 (12), 0 (0)
Implementation	1	8 (1) 100 (13), 0 (0)	8 (1.5) 100 (13), 0 (0)	8 (1.5) 84.6 (11), 0 (0)
Data extraction	1	8 (1) 84.6 (11), 0 (0)	8 (0.5) 92.3 (12), 7.7 (1)	8 (1.5) 92.3 (12), 0 (0)
Cautions	1	8 (1) 84.6 (11), 0 (0)	8 (1) 92.3 (12), 7.7 (1)	7 **(5)** **53.8 (7), 23.1 (3)**
	2	8.5 (1.3) 85.7 (12), 0 (0)	8.5 (1) 100 (14), 0 (0)	8 (1) 100 (14), 0 (0)
Recommended use	1	8 (1.5) 100 (13), 0 (0)	8 (2) 92.3 (12), 0 (0)	9 (1) 92.3 (12), 0 (0)
Non-recommended use	1	8 **(3.5)** **69.2** (9), 7.7 (1)	9 (1) 92.3 (12), 0 (0)	8 **(3.5)** **69.2** (9), 7.7 (1)
	2	8 (2) 85.7 (12), 0 (0)	8.5 (1) 100 (14), 0 (0)	8 (2.3) 78.6 (11), 7.1 (1)
To report	1	9 (1) 100 (13), 0 (0)	8 (1) 92.3 (12), 0 (0)	8 (1.5) 92.3 (12), 0 (0)
Interpretation		***Regional activation***	***Muscle fiber properties***	***Single motor unit activity***
General considerations		8 (1.5) 84.6 (11), 0 (0)	8 (1.5) 76.9 (10), 7.7 (1)	8 (1.5) 92.3 (12), 0 (0)
Cautions		8 (1.5) 92.3 (12), 0 (0)	8 (1) 100 (13), 0 (0)	8 (1) 92.3 (12), 0 (0)
To report	1	8 (2) 84.6 (11), 0 (0)	8 **(4)** **69.2** (9), 7.7 (1)	8 **(4)** **69.2** (9), 7.7 (1)
	2	8 (1.3) 92.9 (13), 0 (0)	8 (1) 92.9 (13), 0 (0)	8 (1) 100 (14). 0 (0)

*Numbers in bold represent items that did not reach consensus.

FIGURES:

Figure 1: Example of HDsEMG signals recorded from the vastus medialis during a ramp-and-hold isometric contraction to a target of 20% of the maximal voluntary torque. Left: HDsEMG electrode configuration with 8x4 electrodes (spaced 10 mm center-to-center). Middle: example of 7 differential EMG signals obtained from the most lateral column of electrodes. Right: 50-ms spoch of the differential signals to show muscle fiber action potentials.