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A Novel Validation Approach for High Density Surface EMG Decomposition in Motor Neuron Disease

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Abstract

This study presents a novel two-source approach for validating the performance of high density surface electromyogram (EMG) decomposition. The approach was developed taking advantage of surface EMG characteristics of amyotrophic lateral sclerosis (ALS). High density surface EMG data from ALS patients can be divided to the sparse dataset and the interference dataset, with the former decomposed by expert visual inspection while the latter independently decomposed by the surface EMG decomposition algorithm. The agreement of the decomposition yields from the two datasets can be quantified for evaluating the surface EMG decomposition performance. The novel validation approach was performed for a recently developed method called automatic progressive FastICA peel-off (APFP) for high density surface EMG decomposition. The APFP framework was used to automatically decompose high density surface EMG signals recorded from the first dorsal interosseous muscle of ALS subjects. The common motor units independently decomposed from the interference dataset and the sparse dataset demonstrated an average matching rate of $99.18\% \pm 1.11\%$. The characteristics of ALS surface EMG also facilitate a step by step illustration of the APFP framework for high density surface EMG decomposition. The novel approach presented in this study can supplement conventional two-source validation for accuracy assessment of decomposed motor units from experimental signals, which is essential for development of surface EMG decomposition methods.

Keywords

Two-source validation; high density surface EMG; automatic decomposition; amyotrophic lateral sclerosis; progressive FastICA peel-off

I. INTRODUCTION

Electromyogram (EMG) decomposition is the process of breaking down the EMG signal into the contributions of its component motor unit action potential (MUAP) trains. It plays a fundamental role for investigation of motor control and examination of neuromuscular diseases. EMG decomposition is based on the observation that MUAPs from different motor units tend to have distinctive waveforms, and the MUAP waveforms from the same motor unit remain almost constant from discharge to discharge, so template matching can be used to sort out different motor units if there is no or little MUAP superposition in the signal. While this can be the case for intramuscular (needle or fine wire) EMG at very low muscle contraction levels, MUAP superposition occurs more frequently with increasing of muscle contraction levels, imposing most challenges for EMG decomposition. Depending on specific applications, previous intramuscular EMG decomposition methods either chose to only sort out those MUAPs without superposition (to obtain MUAP waveform information) or focused on separation of superposed MUAP waveforms (to obtain motor unit discharge information), which is obviously a more challenging task [1–4].

Compared with intramuscular EMG, decomposition of surface EMG is much more difficult because regular surface electrodes (usually centimeter size) provide global muscle activity, and the recorded surface EMG routinely contains a large extent of MUAP superposition (even at low muscle contraction levels). Furthermore, surface recordings of MUAP waveforms from different motor units are less distinguishable compared with intramuscular (needle or fine wire) EMG recordings, due to volume conductor effects of skin and subcutaneous body tissues. Therefore, most template matching or clustering methods that rely on MUAP morphological information are likely not applicable to surface EMG decomposition. In recent years, the development of biological signal amplification technology and surface electrode arrays (comprised of a number of tiny electrode probes with small inter-probe distance) provides a novel approach of high density surface EMG recording [5, 6]. Taking advantage of the spatial information of an electrode array and the unique location of each motor unit within a muscle, two-dimensional MUAP template matching can be applied for surface EMG decomposition [7]. Meanwhile, more advanced and successful approaches of high density surface EMG decomposition have been developed relying on blind source separation techniques, such as the convolution kernel compensation (CKC) and its different variations [8–12], and the recently developed progressive FastICA peel-off (PFP) framework [13, 14].

When a method is proposed to decompose an EMG signal, it is incumbent to prove that the decomposition is performed accurately. Assessment of EMG decomposition performance remains a crucial issue for the development of decomposition methods, especially for a real or experimental EMG signal (whose composition is not known *a priori*). To validate surface

EMG decomposition performance, a “two sources” method has often been used, in which surface EMG and intramuscular EMG signals can be simultaneously recorded [15]. The agreement of the common motor unit discharges independently decomposed from intramuscular EMG (using the already well-established methods) and the surface EMG (using the algorithm to be assessed) can be quantified for evaluating the surface EMG decomposition performance. Such a two-source validation approach has been performed in several previous studies for assessing different surface EMG decomposition methods [16–18].

In this study, we have performed automatic decomposition of high density surface EMG signals of amyotrophic lateral sclerosis (ALS) patients, using a recently developed method called automatic progressive FastICA peel-off (APFP) [13, 14]. Amyotrophic lateral sclerosis is a fatal neurodegenerative disease characterized by progressive motor neuron degeneration. Taking advantage of the surface EMG characteristics of ALS patients, we have developed a novel two-source validation approach for assessing the decomposition accuracy of experimental high density surface EMG signals. Compared to the conventional two-source validation that usually relies on needle insertions, the novel approach does not require invasive intramuscular EMG recording. In addition, it can avoid synchronization of intramuscular and surface EMG devices, which can be complicated due to different sampling rates, even with a trigger signal. This study presents application of the novel validation approach for the APFP framework. Such an approach can also be used to assess other high density surface EMG decomposition methods.

II. METHODS

A. PFP and Its Automatic Implementation

The progressive FastICA peel-off (PFP) framework can be viewed as a process of progressively expanding the set of motor unit spike trains [13]. In the framework, the initial set of motor unit spike trains can be estimated by applying FastICA [19] to high density surface EMG signals [20–22]. Then, a peel-off procedure is employed to subtract the estimated MUAPs of the identified motor units from the original signal. Such a procedure mitigates the effect of the already identified motor units on the FastICA convergence, so more motor units can emerge when processing the residual signals again with FastICA. In order to ensure the reliability of the extracted motor unit spike trains, a constrained FastICA [23] is applied to assess each spike train and correct possible erroneous or missed spikes. The framework is iterative by repeatedly running these procedures until no more new motor units emerge.

Automatic decomposition is of particular importance for promoting application of high density surface EMG in both research and particularly clinical laboratories, while the above PFP framework often requires human operator interactions regarding extraction of motor unit spike trains from FastICA outputs and reliability judgement of the extracted motor units. To facilitate automatic surface EMG decomposition, we have developed an automatic version of the PFP framework [14]. In automatic PFP (APFP), a series of signal processing techniques, such as iterative threshold setting and valley-seeking clustering [24], are applied to automatically extract spikes from the outputs of both FastICA and constrained FastICA.

And multiple constrain parameters are proposed for final screening of the spike trains according to motor unit firing behavior. A pending set strategy is also applied to smooth the process of automatic decomposition. By integrating all these processing procedures, the APFP framework is able to automatically decompose high density surface EMG signals without any human operator interaction [14].

A detailed description of the PFP or APFP framework can be found in [13, 14]. Briefly, the implementation of the framework includes the following main steps:

Step 1. Initialize the residue as the raw surface EMG data, and the spike trains set $\mathcal{V}$ as null set;

Step 2. Run the parallel FastICA on the extended residue and extract the spike trains;

Step 3. For each spike train obtained in Step 2, repeatedly run constrained FastICA until the iterative threshold converges;

Step 4. For each filtered spike train from Step 3, judge its reliability and store the reliable spike trains to the set $\mathcal{V}$;

Step 5. For each channel of the raw surface EMG data, use all the spike trains of $\mathcal{V}$ to estimate the MUAP waveforms, then subtract these identified MUAP trains from the raw EMG data (i.e. a peel-off procedure);

Step 6. Update the residue, then go to Step 2;

Step 7. Output $\mathcal{V}$ if no reliable spike trains emerge or if the program reaches the preset termination conditions.

B. ALS Data Description

The experimental surface EMG signals used in this study were acquired from 9 subjects with the diagnosis of definite ALS or probable ALS with Laboratory Support (El Escorial criteria [25]). The data recording of the study was approved by the local Institutional Review Board. Before the experiment all the subjects gave their written consent. During the experiment, each subject was comfortably reclined in a supine position or seated in a wheelchair, with the tested hand placed in its natural resting position. Surface EMG signals were recorded from the first dorsal interosseous (FDI) muscles, by a flexible 2-dimensional 64-channel (8×8 , individual recording probe 1.2 mm in diameter, center-to-center distance 4 mm) surface electrode array (TMS International BV, The Netherlands). The reference electrode was located near the elbow. The skin over the tested muscle was prepared with gentle abrasion. After gel preparation, the flexible surface electrode array was attached to the tested muscle with a compatible double-sided adhesive sticker. The electrode array was further secured with a medical tape. In this study, surface EMG signals were recorded from the ALS subjects using a strategy commonly used in clinical EMG examination. We did not apply a standard laboratory load cell setup and require subjects to perform precise muscle force control at different contraction levels. Instead, we provided a resistance force to the examined muscle and instructed the subject to first perform an isometric contraction at a low

force level. The subject was asked to maintain the contraction level for at least 5 seconds. Then the subject was asked to increase the contraction level and maintain the contraction again. Different contraction levels were recorded for each subject. Substantial rest time between trials was provided to prevent potential fatigue during the experiment. A Refa128 amplifier (TMS International BV, The Netherlands) was used to record surface EMG signals. The signals were sampled at 2 kHz per channel, with a band pass filter setting at 10 – 500 Hz.

C. Automatic Decomposition of ALS Signals

The collected high density surface EMG signals from the ALS subjects were automatically decomposed using the APFP framework [14]. For each trial, we terminated the decomposition program after three peel-off iterations. Empirically, running more peel-off iterations may help extraction of a small number of additional motor units, but meanwhile will reduce the program efficiency. As a trade-off, we chose to run three peel-off iterations for the ALS data.

By observing the collected ALS surface EMG signals, we found that for some data trials, the 64 channels of surface EMG show different fullness levels. Some channels contain high levels of MUAP superposition, while some other simultaneously recorded channels of the same electrode array have very sparse signals, due to progressive motor neuron degeneration. These sparse signals typically contain a very small number of distinguishable motor units with large MUAP amplitudes. Since these large MUAPs (likely due to muscle fiber reinnervation) are usually prominent compared with the background small MUAPs or noises, they can be readily discriminated by visual inspection or routine clustering methods. It follows that a comparison of the APFP decomposition of those busy channels that contain high levels of MUAP superposition with the expert visual inspection of the very sparse channels can provide an objective evaluation of the APFP decomposition performance. Therefore, we have developed a novel approach, as described in the next session, to quantitatively evaluate the automatic decomposition performance using the APFP framework.

D. Two-Source (Dataset) Validation

To quantitatively assess the decomposition performance, we examined the surface EMG data and chose the data trials which can apply the above assessment rationale described in Section C. For each of the data trials, we selected different recording channels to form two datasets, i.e. the interference dataset and the sparse dataset. The interference dataset only contained those channels with high levels of MUAP superposition while the sparse dataset only contained those channels capturing distinguishable motor units, readily discriminated through visual inspection or routine clustering algorithms. To decompose the sparse dataset, it was straightforward to determine the firing instants of the motor units with distinctive amplitude. Such motor units are hardly affected by background EMG/noise activity, and can be easily identified through visual inspection. The interference dataset was automatically decomposed by the APFP framework. The decomposition results from the two datasets were then compared. Note that the decompositions of the interference dataset and the sparse dataset were independent with each other, i.e. no any information from one dataset was used

for the decomposition of the other dataset. The decomposition of the interference dataset was completely automatic without any human operator interaction.

To evaluate the extent of agreement in firing instants of the common motor units, independently obtained from the interference dataset and the sparse dataset, the matching rate (MR) for each common motor unit from these two datasets was calculated as [26]:

$$MR = \frac{2 \cdot N_{COM}}{N_I + N_S} \cdot 100\%$$

where N_{COM} stands for the number of spikes of a motor unit identified from both the interference and the sparse datasets. N_I and N_S are the number of spikes identified from the interference dataset and the sparse dataset, respectively. Two spikes from different spike trains were accepted to be corresponding spikes only when they were located within ± 1 ms. Two spike trains were considered to be common spike trains only when their matching rate was higher than 80%. To further understand how the decomposition performance varied when processing the interference dataset, we also calculated the matching rate of the common motor units identified from both interference dataset and the whole electrode array channels.

Given the high confidence of the identified spike trains from the sparse dataset, they can be considered as standard spike trains. Then, we calculated the false negative rate (FNR) [27] and the false discovery rate (FDR) [28] to further understand how the mismatches occurred in a matching pair (between the interference and sparse datasets). As defined below, the two parameters can be used to measure the proportion of “missing” and “adding” spikes introduced by the APFP framework, respectively.

$$FNR = \frac{N_S - N_{COM}}{N_S} \cdot 100\%$$

$$FDR = \frac{N_I - N_{COM}}{N_I} \cdot 100\%$$

where N_{COM} , N_I and N_S have the same definition as used for calculation of matching rate.

III. RESULTS

Automatic decomposition using the APFP was performed on a total number of 84 trials of electrode array surface EMG signals, collected from the ALS subjects. On average, 9.1 ± 3.3 motor units were extracted from each trial using all the channels of the electrode array.

Figure 1 shows a representative example of the APFP decomposition process using an ALS surface EMG signal. The whole decomposition process is comprised of 3 peel-off steps and each of them is shown in the figure. One channel of the electrode array EMG is used for

demonstration. For each step, the original signal, the estimated MUAP waveforms and firing instants of all the identified motor units (after this peel-off step), the reconstructed signal, and the residual signal are shown from top to bottom, respectively. For an easy comparison the original surface EMG signal is shown in each of the peel-off steps. It can be observed that the original signal demonstrates an interference pattern with obvious MUAP superposition. After the 1st peel-off step, five motor units were identified. The residual signal (after subtracting the reconstructed signal from the original signal) became less superposed than the original signal. Then we continued to search more motor units from the residual signal. After the 2nd peel-off step, nine motor units were identified. The residual signal became more sparse. Simply from visual inspection, we can observe that one large amplitude motor unit, which was masked by superposition and difficult to reveal from the original signal, started to show up. This motor unit was approved to be the 10th one identified during the 3rd peel-off step. In total, thirteen motor units were identified after the 3rd peel-off step, and the residual signal was very small. Note that in every peel-off step, we searched new motor units in the residual signal and used the firing information of all the extracted motor units to estimate their MUAP waveform from the original signal. So the MUAP template waveforms for each motor unit were updated after each peel-off step. With increased number of the extracted motor units, the MUAP waveform estimation would be more accurate. As we can observe from the figure, the extracted MUAP templates of the same motor units remained quite consistent during the three peel-off steps, implying the reliability of the MUAP waveform estimation.

To quantitatively assess the decomposition performance of the APFP, we examined the surface EMG data collected from the ALS subjects and chose 22 trials to apply the two-dataset validation. Overall the number of channels in the interference dataset was 42.6% of the total number of channels. In total, 159 (on average 7.2 ± 3.1 per trial) motor units were identified from these 22 trials by applying the APFP to all the electrode array channels. A total of 136 (on average 6.2 ± 3.5 per trial) motor units were identified from the interference dataset using the APFP and a total of 34 (on average 1.5 ± 0.5 per trial) motor units were identified from the sparse dataset. The decomposition results of the APFP using all the electrode array channels and only using the interference dataset were compared and there were 124 (on average 5.6 ± 3.1 per trial) common motor units with an average matching rate of $99.32\% \pm 1.76\%$. When comparing the decomposition results from the interference dataset and the sparse set dataset, we found that all the 34 motor units from the sparse dataset were also extracted from the interference dataset. For these 34 common motor units, the average matching rate (MR) was $99.18\% \pm 1.11\%$; the average false negative rate (FNR) was $0.75\% \pm 1.25\%$; the average false discovery rate (FDR) was $0.85\% \pm 1.28\%$.

Figure 2 shows an example of the validation process, where the top panel is the selected channels from the interference dataset, the middle panel is the firing instants of the identified motor units using the APFP decomposition, and the bottom panel shows two channels from the sparse dataset. In this example, the interference dataset contains 14 channels and for clarity only three channels are shown in the figure. It was straightforward to extract two different motor units from the two channels of the sparse dataset by visual inspection of the action potential amplitude and waveform information. These two motor units were also obtained by applying the APFP to the 14 channels' of interference dataset (indicated by red

and green bars). The firing instants of these two common motor units from the two different datasets matched very well, as shown in the figure, where the same color (red or green) was used to represent the common motor unit from the interference dataset and the sparse dataset.

IV. DISCUSSION

We have performed automatic decomposition of high density surface EMG signals from ALS patients using a previously developed method called APFP [14]. The contribution of this study mainly lies in the development of a novel validation approach for assessing the decomposed motor units from experimental surface EMG signals. Assessment of decomposition accuracy is essential for an EMG decomposition study. The most common method for assessment of decomposition performance is to simulate an EMG signal [29–32], during which the input components can be controlled so that the decomposition performance under different conditions can be quantitatively analyzed. However, the reliability of the simulation based assessment to a large extent depends on the realism of the simulation, which can be close but not equivalent to experimental EMG. For a real or experimental EMG signal, the composition is not known *a priori*. One evaluation approach is to compare the decomposition result of the computer based methods with the visual judgement of an experienced human operator. The expert's judgment can usually be trusted when there is no or little MUAP superposition contained in the EMG signal. This approach is often used for intramuscular EMG decomposition, typically at low muscle contraction levels. With increasing MUAP superposition or contraction levels, it becomes difficult to rely on visual judgement for assessment of decomposition performance.

To validate surface EMG decomposition performance, a so called “two-source” method has often been used, which typically involves simultaneous surface EMG and intramuscular EMG recordings [15]. The intramuscular EMG signal can be decomposed using already well-developed methods, and the surface EMG can be independently decomposed using the designed method (to be evaluated). It is expected that we may find motor units contributing to both signals. Cross-checking of the timing information (i.e. agreement on the timing) of the common motor unit discharges will provide an estimation of the decomposition accuracy. The underlying rationale is that if the independent decomposition of two separate signals agrees on the timing of a particular motor unit discharge, most likely they are both correct. Otherwise, they would both have to involve an exactly the same amount of error. The probability of this situation arising is very small. The conventional two-source validation usually involves invasive intramuscular EMG recording. An alternative two-source validation is to solely record surface electrode array EMG and independently decompose different channel subgroups of the array using the designed methods and cross-check the timing information [11]. A limitation of this approach is that applying the same method to different datasets tends to increase the possibility of inducing similar errors, thus compromising the validation.

In the current study, we have developed a novel two-source validation method for assessment of high density surface EMG decomposition. The key design was to take advantage of surface EMG characteristics of ALS patients and the spatial recording capacity

of an electrode array. Due to progressive motor neuron degeneration and muscle fiber reinnervation, surface EMG signals of ALS can be more sparse than those signals from neurologically intact subjects. For electrode array surface EMG data of ALS, it is possible to select channels to form two groups: the channels with a considerable amount of MUAP superposition (i.e. the interference dataset) and the very sparse channels (i.e. the sparse dataset) containing only 1 or 2 large amplitude motor units (possibly due to muscle fiber reinnervation). The one or two motor units of the sparse dataset are readily discriminated from the background signals with high confidence by either visual judgement or conventional thresholding or clustering methods. The EMG channels in the interference dataset with considerable MUAP superposition can be independently processed by the surface EMG decomposition method to be evaluated. Similar to the conventional two-source validation, cross-checking of the timing information of the common motor unit discharges independently obtained from each of the two datasets will provide an estimation of the decomposition accuracy.

The novel validation approach was illustrated by applying it to the APFP framework. The APFP was a recently developed method for high density surface decomposition [14]. The performance of the APFP has been first assessed by a simulation approach [13, 14]. We have also independently applied the PFP and the CKC methods to decompose the same sets of experimental high density surface EMG signals, and achieved high degree of agreement for the extracted common motor units, thus providing supportive evidence of the decomposition accuracy for both methods [26]. Taking advantage of the ALS surface EMG data, in this study we have performed a novel two-source validation of the APFP framework. High matching rates were obtained for the common motor units independently decomposed from the interference dataset and the sparse dataset. Note that the decomposition of the interference dataset was performed automatically using the APFP without any human operator interaction. The outcomes from this novel two-source validation indicate that the APFP can reliably decompose at least a subset of motor units from the processed electrode array surface EMG signals. Given the fact that every channel in the interference dataset contains a considerable amount of MUAP superposition, the outcomes also highlight the capability of the blind source separation techniques for dealing with a rather difficult situation for conventional template matching based methods. In other words, after removing all the relatively sparse channels, the APFP can still reliably extract motor units from those channels with heavy MUAP superposition.

To describe the nature of the mismatched spikes of the common motor units, we have further calculated the false negative rate and the false discovery rate. The results suggest that the mismatches are not always from “missing” or “adding” spikes, i.e. the mismatches are actually from both situations. Note that for the sparse dataset, because there is little or no MUAP superposition contained in the signal, single motor units can be discriminated with high confidence. However, this does not necessarily imply that the decomposition result from the sparse dataset is 100 percent accurate. It follows that the inconsistency in discharge instants of the common motor units does not necessarily mean the decomposition of the interference dataset using the APFP is wrong.

In addition to a comparison between the interference dataset and the sparse dataset, we have also compared the decomposition yield of the APFP using all the electrode array channels and only using the interference channels, respectively. A majority of the motor units from the two decompositions are common ones with a high average matching rate, suggesting the APFP's stable and reproducible decomposition performance. Note that although the average number of interference channels was less than half of the electrode array channels, the number of the decomposed motor units from the interference channels was only slightly reduced compared with that from all the electrode array channels.

Compared with the conventional two-source validation, the novel approach presented in this study has several advantages. For example, the conventional two-source validation usually involves invasive intramuscular EMG recording. Synchronization of the intramuscular and surface EMG devices is also required, which can be complicated even with a trigger signal due to different sampling rates used for intramuscular and surface EMG recordings. By contrast, the protocol for the novel validation approach is noninvasive. No extra effort is needed to perform synchronization of the interference and sparse datasets. In addition, given motor unit loss and muscle fiber reinnervation of ALS patients, it is possible to find the sparse dataset even in relatively high muscle contraction levels. Thus, compared with the conventional two-source validation, the novel approach can be performed during a subject's higher muscle contraction levels (though not necessarily higher MUAP superposition levels).

Nonetheless, the limitations of the novel validation approach should be acknowledged. Like the conventional two-source validation, the novel approach presented in this study provides a direct evaluation only for the common motor units, which account for a small portion of motor units extracted from the interference dataset. In fact, for experimental surface EMG signals there is hardly a standard or solution for direct evaluation of all the decomposed motor units. In this study, taking advantage of the ALS data, we provided a step by step demonstration of the peel-off procedures to illustrate how all the decomposed motor units were obtained. Assuming that the estimated firing instants and MUAP waveforms of the subtracted motor units are correct, then the residual signal tends to become more and more sparse (and ultimately close to background baseline), where the superposed MUAPs can be gradually detangled and start to emerge. Although this is the pattern we can observe from the processed ALS data (such as in Figure 1), it still remains an issue to be solved how to provide a direct and quantitative evaluation for all the decomposed motor units from experimental signals.

In the previous two-source validation studies [16–18], a standard research laboratory load cell setup was usually applied and the subjects were required to perform precise muscle force control or follow a strict trapezoidal force protocol at different contraction levels. Such setup and protocols are difficult to perform in a clinical EMG laboratory. In addition, precise force control may be difficult for patients with neuromuscular disorders even with biofeedback. With the view towards promoting clinical application of high density surface EMG, in this study we have applied a strategy or protocol more mimic to clinical EMG examination (by providing a resistance force to the examined muscle and instructing the subject to perform a series of isometric contractions). Although conventional surface EMG is considered unacceptable as a clinical tool in the diagnosis of neuromuscular diseases [33–

35], recent development of high density surface EMG has been emerging as a promising tool in clinical neurophysiology [5, 36, 37]. High density surface EMG can offer valuable information (e.g., muscle fiber conduction velocity, motor unit territory, innervation zone localization) to supplement needle EMG [36] [37]. The advance in high density surface EMG decomposition will further promote its application in research and clinical practice.

In conclusion, taking advantage of surface EMG characteristics of ALS, this study presents a novel two-source approach for assessing the surface EMG decomposition performance, and demonstrates its application to the APFP, a recently developed framework for high density surface EMG decomposition. The novel validation approach can serve as a supplement to the conventional two-source methods for assessing the accuracy of the decomposed motor units from experimental surface EMG signals, which is required in development of surface EMG decomposition methods.

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Fig. 1.

A typical example with step by step demonstration of the APFP decomposition of electrode array surface EMG signals of ALS subjects. For each of the three peel-off steps, the original signal (from one channel of the electrode array), the estimated MUAP waveform templates, the firing instants of the identified motor units, the reconstructed signal, and the residual signal are shown respectively from top to bottom.

Fig. 2.

A demonstration of the two-source (or dataset) validation (the upper figure) and its zoom in view (the bottom figure). Upper panel: selected channels from the interference dataset; middle panel: the firing instants of the motor units decomposed from the interference dataset using the APFP; bottom panel: two channels from the sparse dataset, where two motor units can be visually identified.