



*Research article*

## **Evaluation method of motor unit number index based on optimal muscle strength combination**

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**Abstract:** Repeatability is an important attribute of motor unit number index (MUNIX) technology. This paper proposes an optimal contraction force combination for MUNIX calculation in an effort to improve the repeatability of this technology. In this study, the surface electromyography (EMG) signals of the biceps brachii muscle of eight healthy subjects were initially recorded with high-density surface electrodes, and the contraction strength was the maximum voluntary contraction force of nine progressive levels. Then, by traversing and comparing the repeatability of MUNIX under various combinations of contraction force, the optimal combination of muscle strength is determined. Finally, calculate MUNIX using the high-density optimal muscle strength weighted average method. The correlation coefficient and the coefficient of variation are utilized to assess repeatability. The results show that when the muscle strength combination is 10, 20, 50 and 70% of the maximum voluntary contraction force, the repeatability of MUNIX is greatest, and the correlation between MUNIX calculated using this combination of muscle strength and conventional methods is high ( $PCC > 0.99$ ), the repeatability of the MUNIX method improved by 11.5–23.8%. The results indicate that the repeatability of MUNIX differs for various combinations of muscle strength and that MUNIX, which is measured with a smaller number and lower-level contractility, has greater repeatability.

**Keywords:** motor unit number index; repeatability; muscle contraction force; coefficient of variation; high-density surface electrode

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### **1. Introduction**

In recent years, the incidence of motor neuron disease (MND) has remained high, attracting a

great deal of attention from domestic and international scholars [1,2]. The clinical manifestations of the disease are upper and lower motor neuron damage, resulting in gradual atrophy of the muscles of the limbs, trunk, chest and abdomen, and the number of motor units in the muscles will gradually decrease with the progression of the disease [3,4]. To assess the progression of the disease, Nandedkar's team proposed the MUNIX technology, which provides a neurophysiological index related to the number of motor units in the muscle: MUNIX, by detecting changes in the index to characterize the trend of the number of motor units over time [5]. MUNIX is calculated using the compound muscle action potential (CMAP) signal and the surface interferential pattern (SIP) signal recorded during voluntary muscle contraction [6]. Compared to the conventional Motor Unit Number Estimation (MUNE) method, this method has the advantages of being non-invasive, fast and easy operation [7–9]. Multiple studies have validated MUNIX as a biomarker for tracking the progression of various neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) [10–13].

Repeatability is the primary condition that needs to be met for the motor unit number index evaluation technique. The method's repeatability is high and retest results are stable, providing a reliable basis for disease diagnosis [14]. The National Institute of Standards and Technology defines repeatability as the dispersion of a variable in repeated measurements under the same experimental conditions (same measurement steps, observers, locations, measuring instruments and short periods of time). To evaluate the repeatability of test methods, two indicators, coefficient of variation (CV) and correlation coefficient (CC), are usually used. Some researchers have measured MUNIX in healthy individuals [10,15,16] and neuronal disease patients [17–19], respectively. The results revealed that the average MUNIX in disease patients was lower than that in healthy subjects and that the average value decreased as the disease progressed, indicating that MUNIX is meaningful for disease assessment. In healthy and neuromuscular disease patients, however, although the CC value used to evaluate reproducibility is normal, the CV values are higher, especially in ALS patients, where they can reach as high as 69.9% in some patients [15,18,20,21]. The phenomenon demonstrates that the repeatability of MUNIX is worse in neurological disease patients, compared with healthy controls, and that the CV index is affected by the health status of the tested subjects. Therefore, there is a need to enhance the MUNIX experimental method to further improve its reproducibility and reduce the negative effects of the experiment itself.

To achieve this, it is necessary to overcome the disadvantageous factors in the experimental method that results in poor reproducibility of MUNIX. The accuracy of the measured CMAP amplitude is essential for ensuring good repeatability of MUNIX [15,17]. When the operator acquires the CMAP, the optimal electrical stimulation location may not be identified, making it difficult to obtain the optimal CMAP signal, resulting in poor MUNIX reproducibility [14]. The development of high-density surface electromyography (HD-sEMG) measurement technology allows for the recording of more comprehensive EMG information in specific muscle areas, providing a powerful tool for obtaining better CMAP signals [22–24]. In addition, the random selection of SIP data segments is also one of the sources of error [25]. The calculation of the motor unit number index depends on the SIP signals extracted under different contractility levels [26], and the degree of activation of motor units is different for different muscle strength levels [27]. Currently, the contractility level used to extract the SIP data segment is still subjectively selected, and there is no literature indicating which levels of contractile force produce more reliable results.

To improve the problem of poor reproducibility of MUNIX technology in patients with neurological diseases, in this study, we used high-density surface electromyography to investigate the

effect of the level and number of contractile forces on the repeatability of MUNIX, and to identify a muscle strength combination, the MUNIX calculated by this combination having the highest repeatability. In particular, combined with HD-sEMG signals, the MUNIX under various contractile force combinations is traversed and calculated, and the best muscle force combination corresponding to the MUNIX with the highest repeatability is chosen. Subsequently, the best muscle force signals from multiple channels were weighted and averaged. Compared with the traditional calculation method, the improved MUNIX method only needs four muscle forces, the MUNIX coefficient of variation is reduced by 11.5–23.8%, and the repeatability of the method is improved.

This paper's main contributions are summarized as follows.

(1) We investigated the effect of different muscle force combinations on the repeatability of MUNIX by iteratively comparing the values of MUNIX and their coefficients of variation under different contractile force combinations, and selected the best muscle force combination for MUNIX calculation.

(2) A weighting method based on the best grade SIP signal was proposed, and Pearson correlation coefficient and bilateral t-test were selected for statistical analysis to further improve the repeatability of MUNIX.

(3) The results of the MUNIX calculation are fast and accurate, with the same reproducibility compared with the latest research, the number of contraction forces used here is less, which eliminates the difficulty of making multiple levels of contraction force repeatedly for patients with muscle weakness and is better tolerated by the subjects.

## 2. Related work

### 2.1. Evaluation of the number of movement units

There are two primary methods of motor unit assessment, i.e., the MUNE and the MUNIX [28]. The MUNIX does not indicate the specific number of motor units in a muscle, but has a strong correlation with the number of motor units in a given muscle. The MUNIX assessment technique is simpler and more reproducible than the MUNE technique and is commonly used clinically to assess the progression of the neuronal disease [29–31]. Common neuronal diseases include ALS [21], progressive medullary paralysis [1], primary lateral sclerosis and progressive myasthenia gravis [29].

### 2.2. Repeatability

Reproducibility is an essential attribute of MUNIX evaluation methods. Improving the reproducibility of MUNIX methods has gradually become a matter of intense interest. Escorcio-Bezerra et al. found that averaging multiple sets of MUNIX measurements of the same muscle resulted in better reproducibility than measuring a single set of MUNIX [32]. Ahn employed digital handheld instruments for recording muscle contraction signals, thereby improving the repeatability of MUNIX by precisely grading muscle contraction force [33]. Peng et al. observed that extracting more SIP signal segments in lower grade contraction force improved the repeatability of MUNIX to some extent [25].

### 2.3. Artificial intelligence in motor unit assessment

With the diagnostic needs of clinical medicine and the rapid development of artificial intelligence (AI) technology, researchers have proposed different surface EMG signal decomposition algorithms [34–37], which are capable of decomposing motor units from the signal and obtaining the electrical activity of motor units, and then diagnosing and treating motor neuron diseases such as stroke and hemiplegic stroke. Hamid et al. proposed an enhanced AI algorithm, which can reliably decompose the surface EMG of isometric contractile forces [36]. Holobar et al. proposed a convolutional kernel offset algorithm based on convolutional blind source separation technique [38], to decompose HD-sEMG signals using a transient linear mixed model [39], and by extracting individual motor unit (MU) activity information to evaluate the patient's health function. More research has been widely developed in biomedical signal processing [40,41] and application scenarios [42].

## 3. Materials and methods

### 3.1. Experimental data collection

This study requires the acquisition of HD-sEMG signals on the dominant biceps muscle of healthy subjects, ensuring the subjects are seated comfortably in a chair, and sequentially acquiring composite muscle action potential signals and varying levels of surface interference pattern signals [6]. The operator stimulates the musculocutaneous nerve of the subject with equally spaced currents, locate and record the stimulation site with the highest CMAP signal response. The subjects were then instructed to perform various levels of maximal voluntary contraction (MVC), and the MVC signal was used to extract the surface interference pattern signal [15,25].

### 3.2. MUNIX calculation method

Before calculating MUNIX, it is necessary to extract SIP data segments from the EMG signals of each contraction class in order to build the SIP data pool. In accordance with the SIP inclusion criteria described by Nandedkar's team, nine levels of EMG signals were sampled for each group in the experiment [6], ten SIP data segments were selected at medium intervals for each level of muscle strength, and the duration of each data segment was 300 ms (614 samples), and a total of  $3 \times 9 \times 10$  SIP data segments were obtained from three sets of experimental data. It should be noted that the duration of maximal voluntary contraction is relatively short in both healthy and diseased subjects, so only five SIP data segments can be extracted at 100% MVC. SIP is copied once, and 10 SIP data segments are obtained.

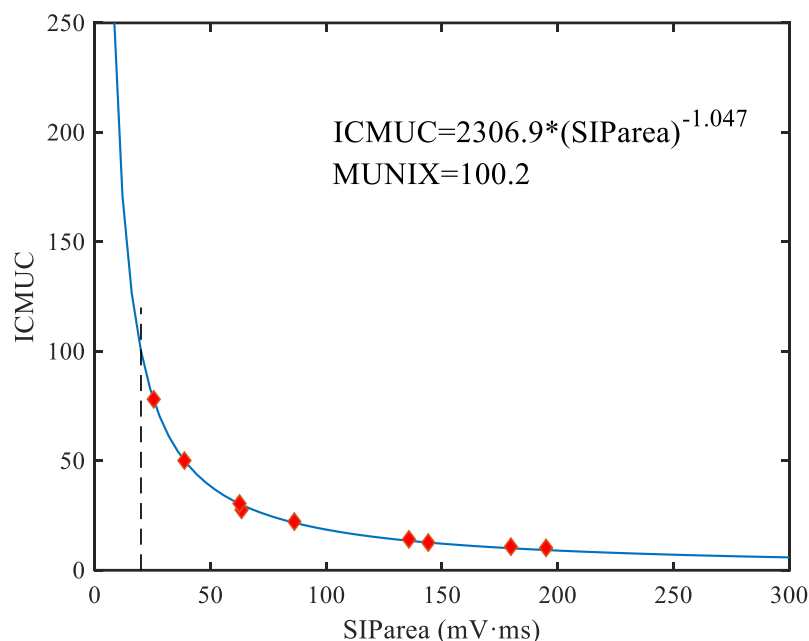
After dividing the SIP signal segment, one SIP data segment from each of the nine levels of MVC is chosen at random. In order to calculate MUNIX, the nine distinct SIP data segments are combined, resulting in  $3 \times 9 \times 10$  SIP data segments,  $3 \times 10$  sets of MUNIX values. Based on the mathematical model developed by Nandedkar's team, which calculates the area (SIParea) and power (SIPpower) of each SIP signal and the area (CMAParea) and power (CMAPpower) of CMAP, the ideal case motor unit count (ICMUC) is calculated as follows:

$$\text{ICMUC} = \frac{\text{CMAPpower} \times \text{SIParea}}{\text{CMAParea} \times \text{SIPpower}} \quad (1)$$

Here, the ideal situation means that the motor units are the same and there is no superposition of motor unit potentials in the SIP signal. Next, conduct power regression fitting and modeling analysis on the entirety of ICMUC and SIParea, and obtain the following relationship:

$$\text{ICMUC} = A \times (\text{SIParea})^\alpha, \quad (2)$$

where  $A$  and  $\alpha$  are values resulting from the ICMUC and SIParea regression fits, respectively. When  $\text{SIParea} = 20 \text{ mV}\cdot\text{ms}$ , it reflects the response of all low-threshold motor units, which is just the approximate area of the CMAP signal, and the ICMUC at this time is MUNIX [5,6]. An example plot of the fitted curves for ICMUC and SIParea is shown in Figure 1, 9-level SIP signal and one CMAP signal fit the MUNIX value.



**Figure 1.** Fitting curve of ICMUC and SIParea.

#### 4. Signal acquisition and analysis

##### 4.1. Measurement of EMG signals

This experimental protocol is based on previous studies [15,25,43]. Eight healthy subjects with no neurological history were recruited (average age  $27 \pm 4$ , 6 males and 2 females), and the biceps brachii of the commonly used arm was selected for MUNIX measurement, which is one of the muscles commonly used for MUNIX measurement [10,44]. Before the experiment, the skin was wiped with medical alcohol to remove dandruff, and the study used flexible  $8 \times 8$  high-density grid electrodes with an electrode diameter of 4.5 mm and an electrode spacing of 8.5 mm, as shown in Figure 2.



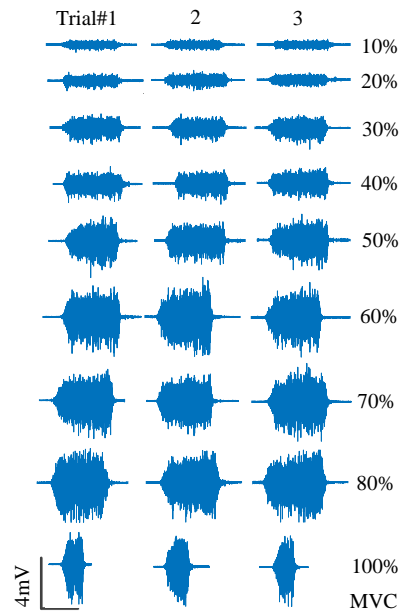
**Figure 2.** High-density surface array electrodes.

Two high-density grid electrodes were adhered adjacent to the muscle belly in the direction of muscle fibers, and then connected to a 136-channel Refa amplifier to collect surface EMG signals at 2048 Hz. The reference electrode is positioned over the elbow of one arm, while the ground electrode is positioned at the wrist of the opposite arm. The subject seated comfortably in an adjustable Biodex chair (Biodex, Shirley, NY) with the dominant arm and wrist secured within the fiberglass cast, minimizing interference from other muscles.

Next, the compound muscle action potential signal and the surface EMG interference phase signal is measured. Initially, a DS7 current stimulator (Digitimer Ltd, Welwyn Garden city, United Kingdom) was used to stimulate the proximal musculocutaneous nerve of the arm. The stimulation current is a pulse with a width of 0.2 ms. Adjusting the position of the stimulating electrode to find the stimulation site with the greatest CMAP amplitude, and then increasing the stimulation intensity at that site in 5 mA increments until the CMAP amplitude no longer increases [34,45], recording the data, and selecting the CMAP signal with the largest negative peak for MUNIX calculations [46]. After electrically stimulating the muscle nerve, the subjects performed three groups of isometric elbow flexion exercises at varying levels of strength, with the strength levels being 10, 20, 30, 40, 50, 60, 70, 80 and 100% of the MVC [25], respectively. In order to control the stability of the muscle force signals, the surface EMG signals at various contraction levels were fed back to the subject and the operator via a screen monitor. The subject was allowed to sufficiently rest between every two voluntary contractions or stimulations to prevent muscle fatigue.

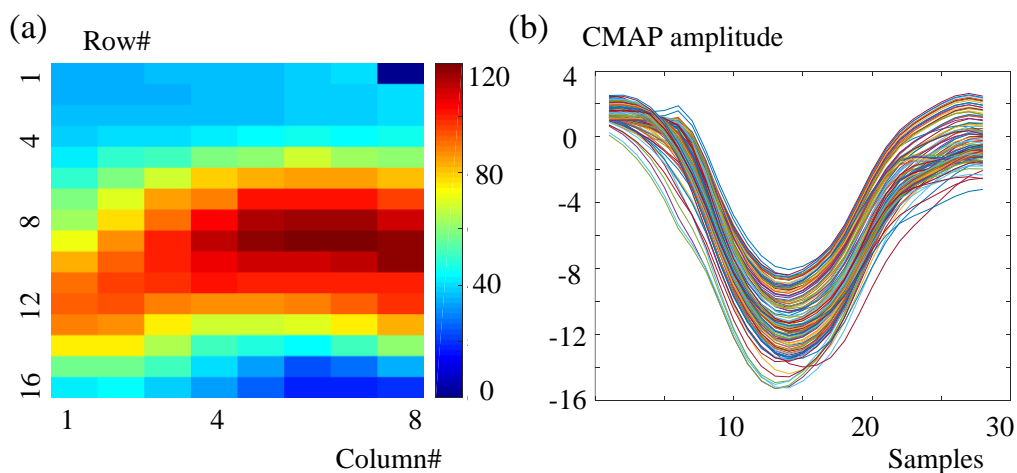
#### 4.2. EMG signal preprocessing

Preprocessing of the original signal containing noise interference is required before calculating MUNIX. First, the baseline drift of all signals is corrected in the time domain using the sliding average method, so that the average signal value is distributed around the baseline 0. Second, for the EMG signals under different levels of contraction movements, a Butterworth filter is used to perform band-pass filtering from 10 to 500 Hz to remove high and low-frequency interference [24,47], and trap filtering at 60 Hz to remove industrial frequency noise. As shown in Figure 3 for three groups of EMG signals after filtering, each group consists of nine contraction levels.



**Figure 3.** 9 levels of surface EMG signals.

Data were recorded in triplicate for each contractile force. It can be seen from Figure 3 that the lower the voluntary contraction force level of the subject, the lower the measured signal amplitude. With the increase of the contraction force level, the excited nerve fibers increases and the wave crest gradually rises. The CMAP signal was then high-pass filtered at 1 Hz and notch filtered at 60 Hz. Figure 4(a) illustrates the distribution of CMAParea values on two electrodes. There are  $8 \times 16 = 128$  rectangular blocks in the whole thermodynamic map, and each rectangle represents an electrode. There is a dark blue square at the top right of the figure, which represents the reference electrode, and the CMAParea value at that location is 0. On the heat map, the color of the rectangular block gradually turns red from the electrode's edge toward its interior. At the contact position of the two electrodes, the color is at its darkest, representing the highest CMAParea value.

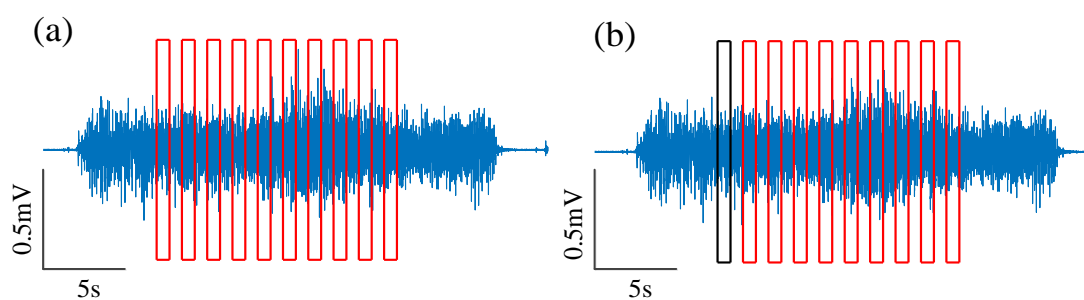


**Figure 4.** CMAParea heat map and CMAP waveform.

Figure 4(b) visualizes the waveform of the 128 electrode CMAP signal. It can be found that the beginning positions of the CMAP signals of each channel are not at the same moment. This is because the two high-density surface electrodes have large areas, it takes a certain time interval for the EMG signal to travel from the location of the motor unit in response to the stimulus to the electrode edge.

#### 4.3. Traversing contraction force combinations and finding the best

After signal preprocessing is completed, the obtained CMAP signal and SIP signal are used for the calculation of MUNIX. The muscle strength class is an essential consideration when constructing the SIP data pool. The conventional method for calculating MUNIX is to extract the SIP signal from five different levels of contraction force, with the contraction force levels of 10, 20 and 50% MVC, and submaximal autonomous contraction force, and maximum autonomous contraction force [6]. As shown in Figure 5(a), 10 SIP data segments divided in 50% MVC for a subject. The level and number of muscle forces used also differed in studies involving the MUNIX method [15,18,48]. Next, HD-sEMG signals were combined with the motor unit number index technique to assess whether the level and number of contractile forces had some intuitive effect on the repeatability of MUNIX.



**Figure 5.** SIP signal segmentation.

The specific process is that the CMAP signal selection method remains unchanged, i.e., the nine levels of SIP data segments utilized the same set of CMAP signals to calculate MUNIX.  $N$ -level strengths ( $N = 3, 4, 5, 6, 7, 8$ ) are sequentially chosen for permutation and combination from the nine levels of muscle force collected previously. There are  $C_9^N$  combinations when  $N$  grades of strength are selected. The MUNIX calculation process under various muscle strength combinations follows the aforementioned MUNIX calculation steps. It should be emphasized that the number of SIP data segments will not have a significant effect on the repeatability of MUNIX [49], and the MUNIX difference calculated by using different SIP segments is within the acceptable range, so under the premise of ensuring the reliability of the results, the way to construct the SIP pool at this time is to select the first SIP data segment from each level of muscle strength, such as depicted in the black rectangular window of Figure 5(b), each group of SIP pools consists of  $1 \times 9$  SIP data segments instead of  $10 \times 9$  SIP data segments, thereby eliminating the need for tedious experimental calculation. Each group of SIP pools in the following still contains  $10 \times 9$  data segments.

Next, using the MUNIX of the channel with the highest CMAP amplitude calculate the coefficient of variation between groups. The coefficient of variation between groups is calculated by dividing the standard deviation of the three MUNIX values groups. On the basis of the average experimental data from 8 subjects, a muscle strength combination corresponding to the smallest coefficient of variation was



chosen, and subsequently compared to the MUNIX values calculated for the nine levels to determine whether the reproducibility of the MUNIX calculated using the best muscle strength was superior.

#### 4.4. Weighted average optimal contraction force

The calculation of the motor unit number index relies on the SIP signal extracted from the muscle strength. In order to further enhance the reproducibility of the MUNIX method, the high-density optimal muscle strength weighted average method is then used to calculate the MUNIX. This strategy is derived from the literature [35,43]. The authors of this paper propose a method for estimating the number of motor units using high-density surface EMG signals. According to Eqs (1) and (2) for calculating MUNIX, SIParea is proportional to MUNIX's value. SIParea is selected as a variable for weighting, and the SIParea signals under the optimal muscle strength in multiple channels are weighted and added. Muscle strength is the 4-grade contractile force chosen previously. Weights are defined first:

$$w(i) = \frac{\text{SIParea}^2(i)}{\sum_{k=1}^m \text{SIParea}^2(k)}, \quad (3)$$

where  $i$  represents the serial number of the electrode channel,  $w(i)$  denotes the weight in each channel,  $m = 2, 4, 8, 16, 32, 64, 128$ , and the denominator part of the formula refers to arranging the amplitudes of the 128 CMAPs in descending order, and taking the square of the SIParea of the corresponding channels of the first  $m$  CMAPs for summation. The MUNIX calculated for each channel is the weighted average of multiple single-channel MUNIX. The denominator part of the formula refers to the 128 channels of CMAP amplitude in descending order, taking the first  $m$  CMAP corresponding to the square of SIParea for summation. MUNIX calculated for  $m$  channels are the weighted average of multiple single-channel MUNIX.

#### 4.5. Statistical analysis

In this paper, the pearson correlation coefficient (PCC) was used to assess the agreement between the MUNIX calculated by the optimal muscle strength method and conventional method. The coefficient of variation between three groups of MUNIX values was calculated in order to characterize the degree of change in MUNIX under different muscle strength combinations levels. A paired Student's t-test was used to determine whether the eight subjects' measurements were statistically different. The difference was considered to be significant at  $p < 0.05$ .

### 5. Results and discussion

The coefficient of variation and correlation coefficient of MUNIX were calculated using 3 sets of experimental data. Table 1 provides a summary of the MUNIX coefficients of variation corresponding to the optimal muscle strength combination for varying numbers of muscle strengths for eight subjects. 10, 20 and 50% MVC were the optimal combinations out of 84 possible combinations when the number of plyometrics was three. The mean value of the coefficient of variation at three levels and four levels of plyometrics was small, and with a difference of only 0.24, corresponding to low plyometrics levels, and the coefficient of variation increased when the number

of plyometrics exceeded four. The selected muscle strength classes with 10, 20, 50 and 70% MVC had the smallest coefficient of variation among all combinations, at 4.68, which was lower than the traditional five muscle strength classes calculated at 5.29, and the coefficient of variation was reduced by 11.53%. All paired student's t-test p-values were less than 0.05, and there was a statistically significant difference between the coefficient of variation of MUNIX with various muscle strength combinations and the conventional method.

**Table 1.** MUNIX coefficients of variation corresponding to the best combinations at different amounts of muscle force.

Sub	Tradit- ional	3strengths	4strengths	5strengths	6strengths	7strengths	8strengths
1	4.39	4.13	3.98	4.25	4.26	4.93	4.86
2	5.66	5.35	4.78	5.31	5.42	5.81	5.91
3	3.93	3.91	3.53	3.73	3.70	4.26	4.15
4	4.12	2.88	3.71	3.95	4.02	4.09	4.78
5	9.07	8.39	7.98	8.64	8.69	9.22	9.01
6	3.35	2.91	2.27	2.38	3.10	3.42	3.72
7	6.74	6.58	6.64	6.34	6.50	6.86	7.01
8	5.12	5.21	4.60	5.33	5.32	5.68	5.71
mean	5.29	4.92	4.68	4.99	5.13	5.53	5.64
<i>p</i>	–	0.039	0.002	0.036	0.026	0.018	0.003
Combination	1258 10	125	1257	12578	12578 10	12567 810	12356 7810

The results of the validation experiment are presented in Table 2, which compares the MUNIX and the intergroup coefficient of variation calculated for 10, 20, 50 and 70% MVC, with the results for the nine levels of muscle strength. The CV values of all eight subjects were reduced to varying degrees, and the MUNIX values for both muscle strength combinations were highly consistent.

**Table 2.** MUNIX and its coefficient of variation calculated for the nine levels of muscle strength and optimal muscle strength combinations.

Sub	1	2	3	4	5	6	7	8
MUNIX1	89.2	91.0	137.7	84.7	108.3	104.5	97.1	104.6
MUNIX2	88.5	90.5	136.3	85.6	108.9	102.4	99.2	101.5
CV1	3.84	2.43	3.33	3.80	9.39	2.98	6.54	3.85
CV2	2.39	2.18	2.85	3.31	8.67	1.84	5.20	2.74

Note: When the mark is 1, 9 levels of muscle strength are used; when the mark is 2, the best combination of muscle strength is used.

Table 3 presents the mean MUNIX values for eight subjects with varying numbers of channels, the mean MUNIX values are the average of the results from the three experiments groups. At the number of channels is 1, it is the MUNIX corresponding to the largest CMAP negative peak channel among 128 channels, and the range is 84.7–137.3. As the number of weighted treated channels

increases, the MUNIX decreases gradually, and the correlation coefficients between the MUNIX under various channel counts and the MUNIX of a single channel are close to 1.

**Table 3.** Mean and standard deviation of MUNIX at different number of channels.

Sub	N=1	2	4	8
1	89.2(2.4)	88.0(2.0)	87.3(1.9)	85.9(2.0)
2	91.0(2.0)	90.9(2.2)	90.8(2.0)	90.1(2.3)
3	137.3(4.2)	137.8(4.4)	134.9(3.0)	133.8(4.6)
4	84.7(3.4)	84.5(2.5)	84.3(2.7)	83.4(2.0)
5	108.3(9.5)	108.2(8.5)	106.8(8.2)	106.6(9.2)
6	103.9(3.3)	103.0(3.8)	101.2(3.0)	100.8(4.5)
7	98.1(6.0)	96.8(5.0)	96.1(5.6)	94.3(5.3)
8	106.2(15.5)	105.9(13.4)	105.0(13.2)	104.5(11.5)
mean	102.3	102.0	101.0	99.9
PCC	–	0.9990	0.9986	0.9979
sub	16	32	64	128
1	84.9(2.2)	84.1(1.9)	81.3(2.4)	74.6(2.8)
2	89.4(2.0)	85.3(1.8)	75.8(1.7)	62.5(1.9)
3	131.5(3.5)	126.4(2.8)	118.3(2.6)	99.7(3.0)
4	84.8(3.2)	82.0(1.9)	72.6(2.2)	62.2(5.4)
5	105.9(8.9)	103.4(9.2)	96.3(9.1)	81.8(8.3)
6	96.1(3.3)	92.8(2.9)	86.0(1.4)	81.6(2.0)
7	93.8(4.6)	91.5(4.1)	83.7(3.8)	77.1(3.3)
8	102.3(14.5)	99.4(13.1)	94.7(11.3)	84.9(8.5)
mean	98.6	95.6	88.8	77.8
PCC	0.9908	0.9905	0.9806	0.9345

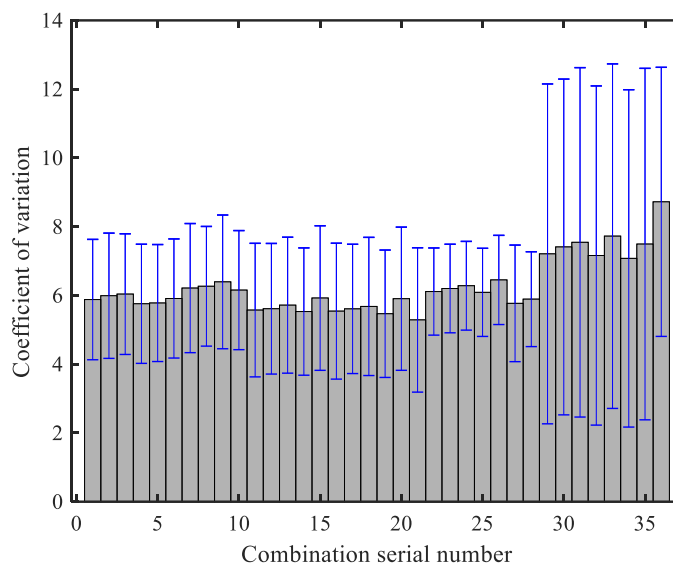
**Table 4.** Coefficient of variation of MUNIX with different number of channels.

Sub	K=1	2	4	8	16	32	64	128
1	2.78	2.25	2.34	2.58	2.25	2.56	2.88	3.29
2	2.37	2.18	1.98	2.10	2.31	2.03	2.07	1.40
3	3.41	3.26	2.82	2.80	2.50	2.59	1.93	1.52
4	3.82	3.35	3.05	2.64	2.73	2.24	3.16	3.67
5	9.10	9.02	8.66	8.71	8.79	9.05	8.87	9.11
6	2.88	2.78	3.01	2.98	2.52	1.69	1.53	1.37
7	6.76	6.34	6.31	6.29	5.74	4.48	3.97	3.62
8	3.99	3.53	3.38	3.23	3.29	3.39	3.06	2.90
mean	4.41	4.08	3.94	3.92	3.77	3.50	3.43	3.36
$p$	–	0.002	0.002	0.011	0.002	0.013	0.022	0.042

In Table 4, the coefficients of variation between groups for the three MUNIX groups with various channel counts are calculated. The CV values decreased gradually as the number of channels increased, as shown in the table, where the mean CV value decreased from 4.41 to 3.36 for the eight subjects, and the coefficient of variation decreased by 23.8%. It is notable that subjects 1 and 4 exhibited an abnormal increase in CV values when 64 and 128-channels were present. CVs with different channel counts were statistically distinct from those with a single channel.

Figure 6 depicts an example error bar graph of the coefficient of variation between the three

groups of MUNIX at seven plyometrics. This graph is the result of averaging the coefficients of variation for eight subjects, and choosing seven of the nine plyometrics for permutation combinations, for a total of 36 plyometric combinations.



**Figure 6.** Coefficient of variation.

In this study, by traversing the repeatability of MUNIX under all muscle force combinations, the muscle force combination corresponding to the best repeatability of MUNIX was identified, and a method of weighting the best muscle force signals of different number of channels was developed, thereby further enhancing the reproducibility of the MUNIX method. In conjunction with the experimental data, it was determined that there was a high degree of agreement between the MUNIX values calculated using the optimal muscle strength combination and the nine muscle strength combinations, and the results of the MUNIX assessment in this study were the same as the previous results in healthy subjects [25,43,50,51], the improved MUNIX method remains reliable.

There has been a gradual increase in research aimed at enhancing the reproducibility of the MUNIX method, which has been optimized in various subjects and experimental settings, yet no definitive solution has been able to solve the problem of poor reproducibility of the MUNIX method. We evaluated the repeatability of MUNIX with different muscle force combinations by combining the previous methods for improving the repeatability of MUNIX, and finally determined 10, 20, 50 and 70% MVC as the best muscle force combinations.

In the calculation of MUNIX, it was found that the correlation coefficients between the different muscle strength combinations in Table 1 and the MUNIX calculated from the traditional five levels of muscle strength were all close to 1, with a high degree of consistency, and similar results were found for other muscle strength combinations, and the MUNIX values were not listed due to space constraints, this phenomenon indicates that the muscle strength combinations do not significantly affect the values of MUNIX, but the coefficients of variation differ. In addition, the results of the optimal muscle strength combination were compared and validated with the calculation of nine levels of muscle strength, and it was discovered that the MUNIX of both were highly correlated, the coefficient of variation of the MUNIX corresponding to the optimal muscle strength combination was reduced, and

the validation experiments demonstrated that the repeatability of the MUNIX calculated using the optimal contractile strength combination was greater.

In comparison to the traditional five muscle strength levels, three levels are identical: 10, 20 and 50% MVC. This paper explains the reason for selecting these lower levels (less than or equal to 50% MVC) of muscle strength from the perspective of the MUNIX mathematical model. Observation of the fitted curves in the preceding Figure 1 reveals that the three lower grades of plyometric force are distributed around the MUNIX definition line, and the SIP data segments selected in the lower grades of contractility have the same motor units and generally do not have a motor unit superposition.

Observing the fitted curves in the Figure 1, it can be determined that the three lower grades of muscle force are distributed near the MUNIX definition line, and the SIP data segments selected in the lower grades of contraction force have the same motor units, and there is generally no superposition of motor units [52], which can meet the definition of “ideal situation” [5]. In contrast, as the muscle force level increases, there will be a greater proportion of motor neurons activated and there will be a significant superposition of motor units, which does not meet the conditions of the definition of ICMUC and reduces the value of ICMUC, which will result in inaccurate calculation results of MUNIX, therefore, only 70% of the maximum voluntary contraction force was chosen as in the high-level contraction force.

Furthermore, Peng et al. demonstrated that muscle force near the defined line had a greater effect on MUNIX assessment than higher-grade muscle force [25], and that extracting more SIP data segments at lower grades of contraction significantly reduced the degree of variability in MUNIX. In Table 1, the combination of muscle force with lower coefficient of variation was found to include 10, 20 and 50% MVC with lower muscle force grades, and this result is in agreement with the findings of Peng et al. [25]. It can be concluded that the lower grade of muscle force near the definition line dominates the value of MUNIX, and the use of the lower grade of contraction force is beneficial to reduce the variation of MUNIX and enhance the reproducibility of the MUNIX method. It should be noted that the best muscle force selected in this study was generated by combining the EMG data of eight healthy subjects, and this combination is superior for the majority of subjects. However, the best muscle force may be different for different subjects' physiques, and to ensure better reproducibility of the MUNIX method, the best muscle force combination or the muscle force selected from the lower grades of contraction can be used.

In a further effort to improve the repeatability of MUNIX, the area of SIP signals with a different number of channels was weighted and summed. It was discovered that the values of MUNIX under a single electrode channel and multiple channels had a strong correlation and were within the normal range [19,53]. Furthermore, the coefficient of variation of MUNIX under multiple channels was significantly reduced. This is due to the fact that multiple electrode channels can incorporate more extensive EMG information, and high amplitude EMG signals are heavily weighted and play a dominant role in the calculation of MUNIX. Nonetheless, in Table 4, two subjects showed anomalies of the increased number of channels and increased coefficient of variation, which may be due to the presence of bio-currents in the human body and interference factors such as coupling crosstalk between adjacent electrodes, which introduced a confounding component in the surface EMG signal, and in the next work of MUNIX evaluation, we can attempt to demix the EMG signal to investigate further for the aforementioned anomalies.

**Table 5.** Comparison of MUNIX and CV with different number of channels.

N	MUNIX-1	CV-1	MUNIX-2	CV-2
1	103.8	4.06	102.3	4.41
2	103.4	3.77	102.0	4.08
4	102.9	3.56	101.0	3.94
8	101.7	3.50	99.9	3.92
16	99.8	3.48	98.6	3.77
32	96.2	3.33	95.6	3.50
64	90.0	3.32	88.8	3.43
128	81.9	3.20	77.8	3.36
strength number	9		4	

To verify the effectiveness of the proposed method, we compared the results with the recently published work in the same area. Nine levels of contraction force were utilized to calculate MUNIX, and the repeatability of MUNIX was enhanced by weighting the CMAP signals of multiple channels. The MUNIX and CV values in each channel were obtained by averaging the data from 8 subjects, as shown in Table 5, the results of MUNIX-1 and CV-1 were calculated by the recent comparative literature method [43], and the results of MUNIX-2 and CV-2 were come from the proposed method. Observing the results under different channels revealed that the MUNIX values derived from the two methods are very close. On the one hand, the accuracy of the MUNIX assessment and the reproducibility of the method in this paper are similar to those of the comparative method, while only four contractile force grades are used in the calculation process, which indicates the proposed method can greatly improve the speed of MUNIX calculation. On the other hand, the number of contractile forces used here is much smaller, which can eliminate the difficulty of repeatedly making multiple grades of contractile forces for patients with muscle weakness, which is more conducive to the maintenance of contractile force This is more conducive to the maintenance and accurate grading of contractility, and reduces the variability of repeated MUNIX measurements.

It should be noted that the limitation of this study is that the improved protocol was not applied to patients with neurological disease, and additional validation is required to determine whether patients have similar findings. This protocol addresses the theoretical aspects of the MUNIX method and is believed to have positive implications for the evaluation of MUNIX in patients. Second, the duration of the SIP signal segment chosen during SIP pool construction is insufficient. In a recent publication [46], guidelines for recording signals in MUNIX experiments are provided, suggesting that the duration of the SIP signal segment intercepted in muscle force is 500 ms, adequate SIP data will provide a more reliable foundation for MUNIX evaluation.

## 6. Conclusions

This study combined HD-sEMG signals to determine at what strengths the MUNIX technique should be performed, thereby providing a solution for improved MUNIX reproducibility, which is crucial for clinically accurate assessment of changes in the number of motor units. The results show that MUNIX values calculated with different contractile force combinations vary less, but the coefficient of variation of MUNIX is affected by the number and grade of muscle forces, and MUNIX measured using fewer and lower grade contractile forces has better reproducibility. In addition, weighted averaging of the best muscle force signals from multiple channels is suggested to improve

the MUNIX method's reproducibility. In view of the extensive AI application in the fields of motor evaluation [54,55] and human-robot interaction [56,57], for future research, we intend to use AI related techniques to rapidly process massive large amounts of EMG data and further optimize the motor assessment.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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