HHS Public Access

Author manuscript

Clin Neurophysiol. Author manuscript; available in PMC 2018 July 01.

Published in final edited form as:

Clin Neurophysiol. 2017 July ; 128(7): 1158–1165. doi:10.1016/j.clinph.2017.03.044.

Improving the Repeatability of Motor Unit Number Index (MUNIX) by Introducing Additional Epochs at Low Contraction Levels

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Abstract

Objective—To evaluate the repeatability of (Motor Unit Number Index) MUNIX under repeatability conditions, specify the origin of variations and provide strategies for quality control.

Methods—MUNIX calculations were performed on the bicep brachii muscles of eight healthy subjects. Negative effect of suboptimal electrode positions on MUNIX accuracy was eliminated by employing the high-density surface electromyography technique. MUNIX procedures that utilized a variety of surface interferential pattern (SIP) epoch recruitment strategies (including the original MUNIX procedure, two proposed improvement strategies and their combinations) were described. For each MUNIX procedure, ten thousands of different SIP pools were constructed by randomly recruiting necessary SIP epochs from a large SIP epoch pool (3 datasets, 9 independent electromyography recordings at different contraction levels per dataset and 10 SIP epochs per recording) and implemented for MUNIX calculation. The repeatability of each MUNIX procedure was assessed by summarizing the resulting MUNIX distribution and compared to investigate the effect of SIP epoch selection strategy on repeatability performance.

Results—SIP epochs selected at lower contraction levels have a stronger influence on the repeatability of MUNIX than those selected at higher contraction levels. MUNIX under repeatability conditions follows a normal distribution and the standard deviation can be significantly reduced by introducing more epochs near the MUNIX definition line.

Conclusions—The MUNIX technique shows an inherent variation attributable to SIP epochs at low contraction levels. It is recommended that more epochs should be sampled at these low contraction levels to improve the repeatability.

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Conflict of Interest

None of the authors have potential conflicts of interest to be disclosed.

Significance—The present study thoroughly documented the inherent variation of MUNIX and the causes, and offered practical solutions to improve the repeatability of MUNIX.

Keywords

Motor unit number index; electromyography; repeatability; bicep brachii; variation

1. Introduction

The motor unit number index (MUNIX) technique provides a novel neurophysiological proxy of the number of functioning motor units (MUs) in a given muscle (Nandedkar et al., 2004, Nandedkar et al., 2010). Compared with motor unit number estimation (MUNE) approaches, MUNIX is simpler, faster and more patient-friendly. In principle, MUNIX is calculated based on a series of surface electromyography (EMG) measurements under different levels of the maximal voluntary contraction (MVC), namely the surface interferential pattern (SIP), together with a surface EMG measurement under the supramaximal electrical stimulation to the nerve, namely the compound muscle action potential (CMAP) (Nandedkar et al., 2010). The ideal case motor unit count (ICMUC) is then derived by: ICMUC = [CMAPpower/CMAParea] × [SIParea/SIPpower], as a function of SIParea, for each SIP using the same CMAP. In a typical MUNIX procedure, ten data points (ICMUC vs. SIParea) are obtained from two separate series of contractions (10%, 25%, 50% MVCs, suboptimal and maximal), subjected to a certain screening criteria. Last, an extrapolation is performed for all remaining data points to evaluate the ICMUC-SIParea curve at SIParea=20mVms (definition line), which is defined as MUNIX.

As a new technique, MUNIX has been assessed for reliability in both healthy subjects (Ahn et al., 2010, Nandedkar et al., 2010, Neuwirth et al., 2011, Kaya et al., 2014, Escorcio-Bezerra et al., 2016) and patients with neurodegenerative conditions such as amyotrophic lateral sclerosis (ALS) (Ahn et al., 2010, Nandedkar et al., 2010, 2011, Fathi et al., 2016). Current studies are mainly focused on the agreement between two (or more) sets of measurements under different conditions such as two visits separated by weeks or months, or on the same condition by different operators (inter-rater reliability). However, the repeatability, which refers to the dispersion of a variable in repeated measurements under the repeatability conditions, defined as “the same measurement procedure, observer, location, measuring instrument used under the same conditions and repetition over a short period of time” according to the National Institute of Standards and Technology (Taylor, 2009), has rarely raised sufficient attention and often been assumed satisfactory unconditionally. Left uncontrolled, a poor repeatability can severely confound the result interpretation.

Repeatability of MUNIX can be affected by a number of factors. A poor repeatability in selecting the optimal electrode location, often caused by operator’s error, can present direct sources of variations. It could be a challenging task even for a trained neurologist to properly identify the optimal electrode location (Neuwirth et al., 2016). Recent advance in high-density surface EMG measurements can properly capture the optimal electrode location (indicated by the largest CMAP response) and provide a tool to eliminate this source of error. Further, MUNIX can be influenced by a number of variables inherent in its

calculation, including variations in EMG recordings (e.g., a 10 second continuous recording) at same contraction levels and in selections of SIP epoch windows from the same recording, as they directly determine the SIP pool construction and thereby affect the extrapolation outcome. In this study, we aim to explore the origin of source of variations caused inherently by the MUNIX technique and offer practical solutions to improve the repeatability of MUNIX.

2. Methods

2.1. Experimental Protocol

Eight healthy subjects (all male, age of 29 ± 4 years) were recruited in this study. The research protocol was approved by the local ethic committee. The biceps brachii muscle was selected as it was considered one most challenging muscle for MUNIX calculation according to Neuwirth et al. (2016). The experimental setup was configured following a previous study (Peng et al., 2016), as shown in Figure 1(a), to record the isometric contraction of the biceps brachii during flexion, while spurious force contributions from other muscles were minimized with the application of a fiberglass cast. Two flexible high-density 8 × 8 surface EMG arrays were applied on the muscle belly along the muscle fiber direction and connected to a 136 channel Refa amplifier (TMSi, Enschede, The Netherlands). Double-sided were used to secure the electrode-tissue contact and conductive gels were applied. The reference electrode was placed above the elbow of the recoding arm while the ground electrode was placed on the contralateral side. Monopolar surface EMG signals were recorded with a sampling frequency of 2048Hz. All recorded data were stored in a personal laptop for offline analysis.

For each subject, the CMAP signal was firstly obtained following an established procedure (Li et al., 2014). The musculocutaneous nerve was stimulated at the supramaximal level with a bipolar stimulating electrode and the DS7 current stimulator (Digitimer Ltd, Welwyn Garden City, United Kingdom). Briefly, an experienced physician performed a few tentative stimuli to find the optimal stimulation site. After that, a series of stimuli was delivered with an increasing intensity from 5 mA until a supramaximal response was reached (in increments of 5 mA). Each stimulus was a rectangular pulse with a width of 200 μs. The supramaximal stimulation was repeated twice and the one with the largest CMAParea was chosen as the source for all subsequent MUNIX calculations. Next, SIP signals were recorded from three complete series of voluntary contractions, each containing eight submaximal contractions at contraction levels from 10% to 80% MVCs in increments of 10% MVC and the 100% MVC. Each contraction period lasted for approximately 12s and was recorded with a visual guidance showing the target contraction force vector in two directions on a computer screen (Peng et al., 2016). In total 27 contraction periods were recorded for each subject, as shown in Figure 2(a), with their orders randomly generated by a computer code and kept blind to the subject. Sufficient recovery time (>15s) was given between consecutive contractions to avoid fatigue. All EMG signals were digitally bandpass filtered (10–500 Hz) according to Zhou et al. (2016) using MATLAB 2014a (The Mathworks, Natick, MA).

Although high-density surface EMG recording is not necessary for the purpose of MUNIX calculation, the characterization of the spatial EMG potentials over the muscle surface (EMG potential mapping) enables a simple and reliable identification of the optimal electrode position (Zhang et al., 2016). Figure 1b shows an example of the high-density CMAP amplitude distribution among all 128 channels. The distribution was stored as a 128 by 1 vector, and the search for the optimal channel was automatically searched with the MATLAB "*max*" function that reported the channel with the largest value.

2.2. Pilot Study – Identify the Critical Contraction Levels

To calculate the MUNIX, SIP epochs were extracted from EMG trials at selected contraction levels, with a length of 300ms (614 data samples). In total 27 SIP epochs (three datasets, nine contractions per dataset, see Figure 2b) were obtained. To reflect the original MUNIX setup as described in Nandedkar et al. (2010), two SIP epochs were sampled from two different and randomly selected (one from each) datasets at each of the five contraction levels. In this case, a total number of 3^5 = 243 equally possible epoch combinations (SIP pools) were generated. Figure 2(b) shows an example of one SIP pool constructed in this manner.

Variation analysis was performed based on the corresponding 243 MUNIX results. The purpose of this pilot study was to assess the contribution of individual contraction levels to the MUNIX variations; therefore, MUNIX results were grouped according to the different epoch combinations at each contraction level, with examples shown in Figure 3(a) and (e). Results of this pilot study should detect whether MUNIX is inherently sensitive to the selection of SIP epochs at certain contraction levels, offering clues to possibly reduce the variation. Analysis of this pilot study was performed using a laptop (Intel i7-4710HQ, 2.5GHz, 32GB memory).

2.3. Construction of Randomized SIP Pools and Original MUNIX

Although the pilot test can properly identify critical contraction levels that account for most MUNIX variations, the limited number of total available SIP epochs makes it inappropriate for examining the proposed strategies for quality improvement to be described in this and next two sections. Alternatively, ten SIP epochs (instead of one) were extracted from each EMG trial at each contraction level (five from the 100% MVC because of a shorter EMG trial length) using a user-coded interactive program that allows the readjustment of boundaries of each SIP epoch to avoid possible artifacts. Figure 2(c) shows an example of a constructed SIP pool in this manner following the original MUNIX setup. This large number of possible combinations should properly accommodate various possibilities during the MUNIX procedure and provide a decent base to avoid the problem of overfitting (erroneously superb repeatability because the total number of possible SIP pools is too small).

The criteria were imposed on SIP epochs according to Nandedkar et al. (2010) to avoid erroneous overestimation of the MUNIX as a result of low SIP amplitudes. The randomized SIP pool construction process was facilitated by generating a combination of random numbers with each digit indicating the source of datasets and SIP epoch number at each

contraction level. Ten thousands of such combinations for each testing scenario detailed in the next two sections were generated and executed in parallel at an AMD Cluster (Maxwell, 8 cores on 2 CPUs, 16 nodes total, 2GB RAM) in the Center for Advanced Computing and Data Systems at the University of Houston.

2.4. First Strategy – Additional SIP Epochs at Low Contraction Levels

Additional (in addition to one) SIP epochs were extracted from each EMG trial at low contraction levels of 10, 30 and 50% MVCs. The reason for selecting these three contraction levels was detailed in Discussion (Section 4.1). For simplicity, let N_5 denote the number of additionally extracted SIP epochs from each EMG trial (the subscript '5' indicates five different contraction levels constituting the SIP pool). The total number of SIP epochs in the constructed SIP pool is: $(N_5 + 1) * 2 * 3$ (number of total epochs per EMG trial, multiplied by two EMG trials per contraction level, and multiplied by three low contraction levels containing additional SIP epochs – 10, 30 and 50% MVCs) plus $1 * 2 * 2$ (one SIP epoch per EMG trial multiplied by two EMG trials per contraction level and multiplied by two contractions levels − 80 and 100% MVCs). Figure 2(c) shows an example of constructed SIP pool when $N_5 = 1$ (red and blue blocks). This strategy was then examined by running a series of tests with different N_5 values (from 0 to 4, with 0 representing the original MUNIX), with the purpose to assess whether introducing more data points near the MUNIX definition line can further reduce the variations. For each N_5, ten thousands of tests were performed.

2.5. Second Strategy - Additional Contraction Levels (Nine Instead of Five)

Another possible solution to stabilize the extrapolation is to introduce extra data points from additional contraction levels (instead of introducing extra data points at the same contraction level as described in the first strategy). The rationale is based on a recent study that recommended the inclusion of SIP epochs at continuous contraction levels (from 10 to 60% MVC, in increments of 10% MVC, plus the 100% MVC) (Kaya et al., 2014). Accordingly, in this step, the SIP pool was constructed by expanding the SIP epochs selections from the original five contraction levels to nine contraction levels (from 10 to 80% MVC in increments of 10% MVC plus the 100% MVC).

Furthermore, we also examined the cases when both strategies were applied. SIP pools were then constructed with extra SIP epochs extracted from EMG trials at low contraction levels (10, 20, 30, 40 and 50% MVCs). As a result, the total number of data points in each SIP pool is: $(N_9 + 1) * 2 * 5 + 1 * 2 * 4$ (N_9 from 0 to 4, with 0 representing the second strategy alone).

2.6. Data Analysis

For each subject, MUNIX values obtained in each scenario was checked for normality using Kolmogorov–Smirnov test. Mean and standard deviations (STD) were reported to describe the MUNIX variations. The range of MUNIX measurements (calculated as the difference between 95% and 5% percentile) was also obtained. The paired two-tailed t-test was employed to detect the significance in reported variables between any two scenarios for all eight subjects, with the significance level established at $p = 0.005$.

3. Results

Two CMAPs were obtained for each subject from high-density surface EMG recordings under the supramaximal stimulation and visualized on a surface contour map, as shown in Figure 1(b). The channel featured with the largest negative peak area (CMAParea) was immediately discernible and coincided between both CMAP recordings for all subjects. This step ensured that the effect of suboptimal electrode position on MUNIX calculation was properly eliminated.

3.1. Pilot Study

Table 1 summarizes the results for the pilot study. Mean MUNIX measurements varied from 84.1 to 152.3 between subjects. Results of all 243 trials were grouped at each contraction level according to the particular combination of selected epochs (two out of three) to evaluate the effect of the contraction level on the MUNIX variations. A clustering effect was observed in the MUNIX distribution, as shown in Figure 3(a) and (e) in two example subjects. The separation between these clusters can be explained by the different epoch combinations in low contraction levels (majorly by 10% and 30% MVCs and partially by 50% MVC). Figure 3(b) and (f) show all 243 extrapolation curves together with 15 data points. Zoomed-in windows show the effect of epochs at low contraction levels on the extrapolation outcome.

Table 2 summarizes the results of the original MUNIX procedure ($N_5 = 0$) and the first strategy (N_5 from 1 to 4). No significant difference was detected between the mean MUNIX values of eight subjects for any pair of N_5 values, while significant decreases were found in the STDs and ranges of the results when the first strategy was applied compared to those when the original MUNIX procedure was applied. Similarly, table 3 summarizes the results of the second strategy ($N_9 = 0$) and the combined strategies (N_9 from 1 to 4). Compared with the results when the original MUNIX procedure was applied, results in table 3 showed no significant differences in the mean MUNIX values but significant decreases in the STDs and ranges.

MUNIX distributions under the repeatability conditions can be well characterized by a normal distribution ($N = 10000$), with a nearly zero value ($p < 0.0001$) of the Kolmogorov–Smirnov test statistic in all scenarios. Figure 4 shows an example of the MUNIX value distribution.

4. Discussion

The quality performance of MUNIX has been assessed in a number of studies. However, the repeatability of MUNIX under repeatability conditions has rarely received sufficient attention. In one study, a “round-robin” setup was performed (Neuwirth et al., 2016), in which twelve raters made two MUNIX measurements of six muscles on the same subject in two sessions separated by one day. A considerate amount of intra-rater coefficient of variance was reported (mean 14.1 ± 4.3%, range 6.6 – 22.9%). In a second study, the authors used the mean of three consecutive measurements (mean-MUNIX) instead of one single measurement (single-MUNIX) (Escorcio-Bezerra et al., 2016). Clear differences between

the mean-MUNIX and the single-MUNIX were identified. In a third study (Fathi et al., 2016), two MUNIX measurements were performed on the same day by the same operator (interval less than 30 minutes) on two muscles. The coefficient of variation, defined as the percentage ratio of absolute difference between two measurements over the mean, reached up to 30% in certain muscles.

It should be noted that, MUNIX, unlike MUNE, does not carry any physiological meaning (Nandedkar et al., 2010) and therefore is often used in longitudinal studies or comparison studies across subjects. From a perspective of quality control, a satisfactory repeatability should first be established; otherwise, there is no evidence to assess whether the observed differences result from the true physiological changes or simply variations. Despite previous studies have suggested an unsatisfactory repeatability of MUNIX, there is a lack of theories and efforts to track their origins, nor to develop practical solutions. Our study represents the first effort to explain the origin of variations of MUNIX. Furthermore, a practical solution is proposed accordingly and verified to benefit future MUNIX applications.

4.1. Pilot Study - Effect of Low Contraction Level Epochs on MUNIX Variations

The pilot study results revealed a clustering phenomenon in MUNIX measurements, as shown in Figure 3, in which MUNIX values were clustered according to the specific combination of SIP epochs at some critical contraction levels. As a result, the overall MUNIX variation was exaggerated even though each cluster showed a relatively narrower range of variations. A further look into the extrapolation outcome revealed that different selections of SIP epochs at low contraction levels dominantly accounted for these variations. These critical contraction levels did not exceed 50% MVC. The underlying mechanism is straightforward – these epochs were located near the MUNIX definition line (SIParea = 20mVms), so their variations can have a stronger influence on the extrapolation outcome evaluated at the MUNIX definition line, compared with those epochs at higher (more remote to the definition line) contraction levels. It should be noted that these critical contraction levels varied across subjects. For example, in subject #7 (Figure 3e), different selections of 10% MVC data points accounted for most of the total MUNIX variation, and those of 30% MVC data points accounted for a less portion of the total variation. However, in subject #1 (Figure 3a), data points at 10% MVC were rejected from the extrapolation because they did not pass the imposed SIP size criteria. As a result, different selections of 30% MVC data points accounted for most of the total MUNIX variation, and those of 50% MVC data points accounted for a less portion of the total variation. Ideally, extra data points should only be introduced at 10% + 30% MVCs for subject #7 and at 30% + 50% for subject #1, in order to optimally improve the procedure. However, doing so would require an intermittent check of the three SIP size criteria during the MUNIX experiment and disobey the advocated clinical convenience of MUNIX. As a result, in this study, all EMG trails at low contraction levels (<= 50% MVCs) were in general considered as targets for extraction of extra data points, as used in the proposed strategies.

4.2. Reduced MUNIX Variations with Implementation of Proposed Strategies

Results in Table 2 and 3 showed that MUNIX variations can be significantly reduced because of additionally included SIP epochs. In comparison, the first strategy ($N_5 = 1$) yields

a lower STD than the second strategy ($N_9 = 0$) with a less amount of total SIP epochs required (mean STD of 5.6 requiring 16 epochs vs. mean STD of 5.8 requiring 18 epochs). Furthermore, as we showed in the previous discussion, higher level contractions have less influence on the final MUNIX calculation, the introduction of additional contraction levels (such as 60% and 70%) may only have limited contribution to the goal of reducing MUNIX variations. Therefore, it is recommended to extract extra SIP epochs (five instead of one) at each contraction of 10, 30 and 50% MVCs, to reduce the STD by half (as shown by the results when $N_5 = 4$).

Although results show that significant decreases in variations were achieved when both strategies were applied,, the combined solution was not ideal as all five contraction levels from 10% to 50% had to be compensated by introducing extra data points, leading to a less efficient allocation of experimental costs (number of SIP epochs to extra).

4.3. Statistical Foundation of the Original MUNIX Technique and Its Implications

In addition, our study reveals that the distribution of MUNIX under the repeatability conditions can be well described by a normal distribution, as shown in Figure 4. This observation can be explained by the randomness of SIP epoch characteristics even if they are extracted from the same EMG trial. This information may be meaningful as it lays a statistical foundation to modifications of the MUNIX technique. For example, Escorcio-Bezerra et al. (2016) reported an improved reproducibility of a modified MUNIX by using the mean of three separate MUNIX measurements (mean-MUNIX) instead of the original single MUNIX measurement (single-MUNIX). According to the central limit theorem, the mean-MUNIX should also follow the normal distribution with a new STD being the original STD divided by the square root of 3 (sample size = 3). This can partially explain the improved reproducibility in this particular longitudinal study (3 months between test and re-test). Users can decide how many separate MUNIX samples should be taken for averaging to control the STD under a desired limit if the mean-MUNIX method is desired.

The proposed strategy in this study may be more advantageous over the mean-MUNIX method in reducing the STD. This can be proved by applying the central limit theorem to the original MUNIX ($N_5 = 0$) with six single MUNIX measurements to be averaged (sample size = 6, at the cost of total 60 SIP epochs for each SIP pool). A new theoretical STD array of $[2.9, 2.1, 3.2, 1.5, 2.9, 3.9, 3.0, 5.2]^T$ can be obtained in this way, which is significantly higher ($p = 0.005$) than the STDs obtained at $N_9 = 4$, in which both strategies were applied and the MUNIX calculation costs were similar (60 vs. 58 SIP epochs). This advantage should be credited to the optimized allocation of experimental costs (SIP epochs) in the first strategy. Such a pattern exists for all reported MUNIX measurements in this study, provided that the central limit theorem could be applied with non-integer sample size, except for solution 2 ($N_9 = 0$). Such an exception further supports the preference of strategy 1 (extra data points at low contraction levels) over strategy 2 (extra data points at additional contraction levels).

4.4. Limitations

One limitation of this study is the lack of data from patients of neurological disorders. Such information may help better delineate the repeatability of the MUNIX technique under different clinical conditions. Nevertheless, as proposed strategy addresses the methodological issues of the traditional MUNIX approach, it should not be limited to healthy subjects and may be beneficial to measurements on patients. Another limitation is that there were only three separate complete datasets for the random SIP pool construction. Ideally more separate datasets would provide a better basis to our purpose; however, the setting presented in this study properly caters to the clinical setup, according to the standard MUNIX procedure (Nandedkar et al., 2010), to evaluate our solutions. More datasets would unnecessarily lengthen the procedure and may further introduce fatigue as an unwanted confounding factor. Finally, the preferred first strategy was only targeted at the original MUNIX technique, of which the definition line is set at SIParea = 20 mVms. Under certain circumstances, MUNIX can be alternatively evaluated at SIParea = 50mVms (Nandedkar et al., 2010) or at a specific percentage of the subject-specific CMAP with the purpose to compensate the underestimation of motor units caused by the reduction in motor unit action potential amplitude (Li et al., 2016, Zhou et al., 2016). Nevertheless, our strategy can be applied for these MUNIX variations equivalently, by introducing additional SIP epochs at contraction levels close to new definition lines.

5. Conclusions

The repeatability of MUNIX is inherently susceptible to a lack of SIP epochs at low contraction levels. The strategy by introducing additional SIP epochs extracted at low contraction levels demonstrated significantly reduced MUNIX variations and offered practical solutions to future MUNIX studies. It is recommended that five SIP epochs (instead of one) should be sampled at each contraction level of 10%, 30% and 50% MVCs, in order to reduce the standard deviation.

Acknowledgments

This work was supported in part by NIH DK082644 and the University of Houston. The authors would like to thank Dr. Xiaoyan Li, Dr. Bo Yao and Mr. Henry Shin from the University of Texas Health Science Center at Houston for assistance in data acquisition, and Chuan Zhang from the University of Houston for useful discussion.

Abbreviations

MUNIX	Motor Unit Number Index
MUNE	Motor Unit Number Estimation
EMG	Electromyography
SIP	Surface Interferential Pattern
ICMUC	Ideal Case Motor Unit Count
CMAP	Compound Muscle Action Potential

MVC Maximal Voluntary Contraction

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Highlights

1. Motor unit number index (MUNIX) under repeatability conditions follows a normal distribution.
2. MUNIX is inherently sensitive to the selection of surface interferential pattern epochs at low contraction levels.
3. Five (instead of one) epochs should be sampled at low contraction levels to improve repeatability.

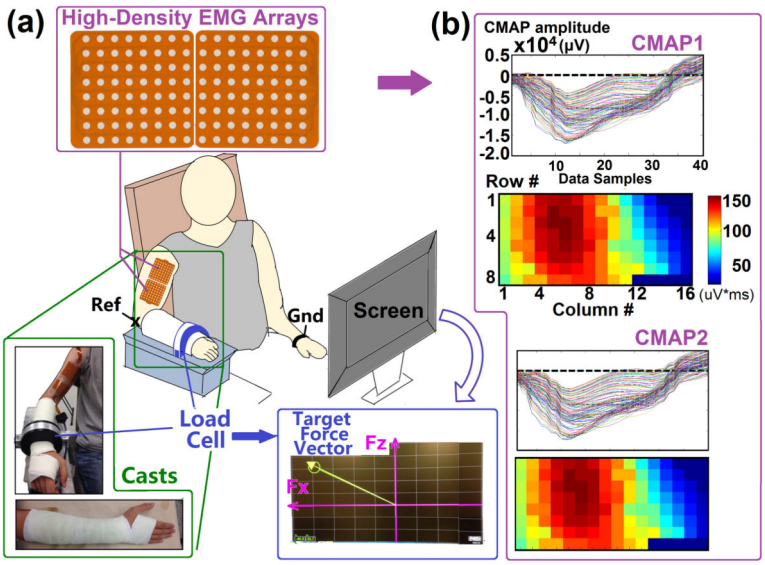

Figure 1.

(a) Illustration of the experimental setup. Constraints were applied to the forearm and wrist to mitigate muscle contractions from non-targeted muscles. A load cell was set inside the mounted ring to provide the visual guidance of the target force vector. Two high-density surface EMG arrays were placed adjacently to cover the surface of the bicep brachii muscle. (b) Example of the CMAP waveforms for all 128 channels and the surface distribution of the calculated CMAParea among the high-density surface for two CMAP repetitions under supramaximal stimulation in one subject (signals in col 12–16, row 8 were zeroed because of apparent movement artifacts caused by a loose contact at the edge). The optimal locations (largest CMAParea), marked with black squares, coincided between two repetitions.

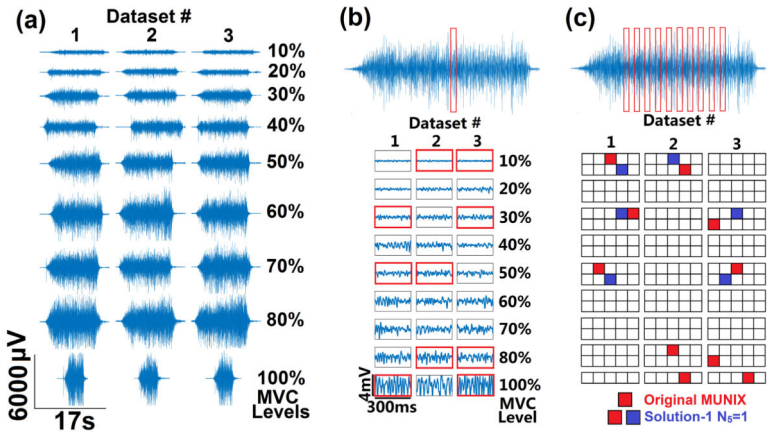

Figure 2.

(a) Example (subject 8) of three sets of EMG signals (dataset 1, 2 and 3) recorded at nine different levels of MVCs (each contraction period lasts for ~12s except MVCs, which last for approximately 5s). (b) Example of SIP pool constructed by drawing only one SIP epoch from each contraction period. An original MUNIX technique SIP pool was constructed by drawing 10 SIP epochs (marked in red rectangles) from two series of contractions (at 10%, 30%, 50%, 80% and 100% MVCs). (c) Example of SIP pool constructed by drawing 10 SIP epochs from each contraction period (5 from 100% MVC). Each square block represents one SIP epoch. All 10 SIP epochs marked in red together shows an example of an original SIP pool. SIP pool for Solution 1 (N_5 = 1) can be constructed by combining both red and blue blocks.

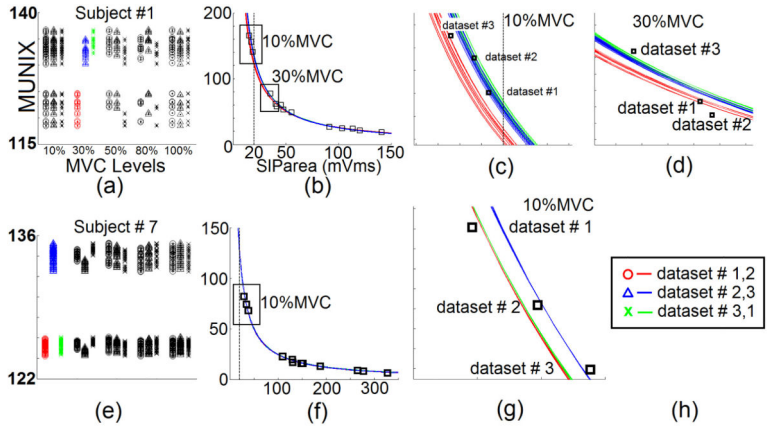

Figure 3.

Contribution of different contraction levels to the MUNIX variations were investigated and illustrated by two example subjects. For each contraction level, the three possible combinations of two epochs randomly selected out of three datasets were marked with a symbol (O, Δ or X), as shown in subplot (h). For example, the combination in Figure 2(b) would be “ΔXOΔX”. At each contraction level, all three symbols together comprise of all 243 MUNIX measurements and therefore the critical contraction level most responsible for MUNIX variation can be assessed by examining the separation of different combinations. For example, in subject #1, it can be concluded from subplot (a) that specific SIP epoch combinations at the 30% MVC dominantly contribute to the MUNIX variations. In this particular example, 10% contraction level has no effect on the MUNIX variations as all three SIP epochs were below 20mVms, therefore excluded from the extrapolation, as shown in subplot(c). In contrast, the specific combination of epochs at 30% MVC largely determines the path of extrapolation curve, as shown in subplot (d). Similarly, for subject #7, SIP epochs at 10% MVC were dominant, as shown in subplot (e–g) due to their proximity to the MUNIX definition line. In both examples, other contraction levels (50% MVC for subject #1 and 30% MVC for subject #7) also partially contributed to the MUNXI variations.

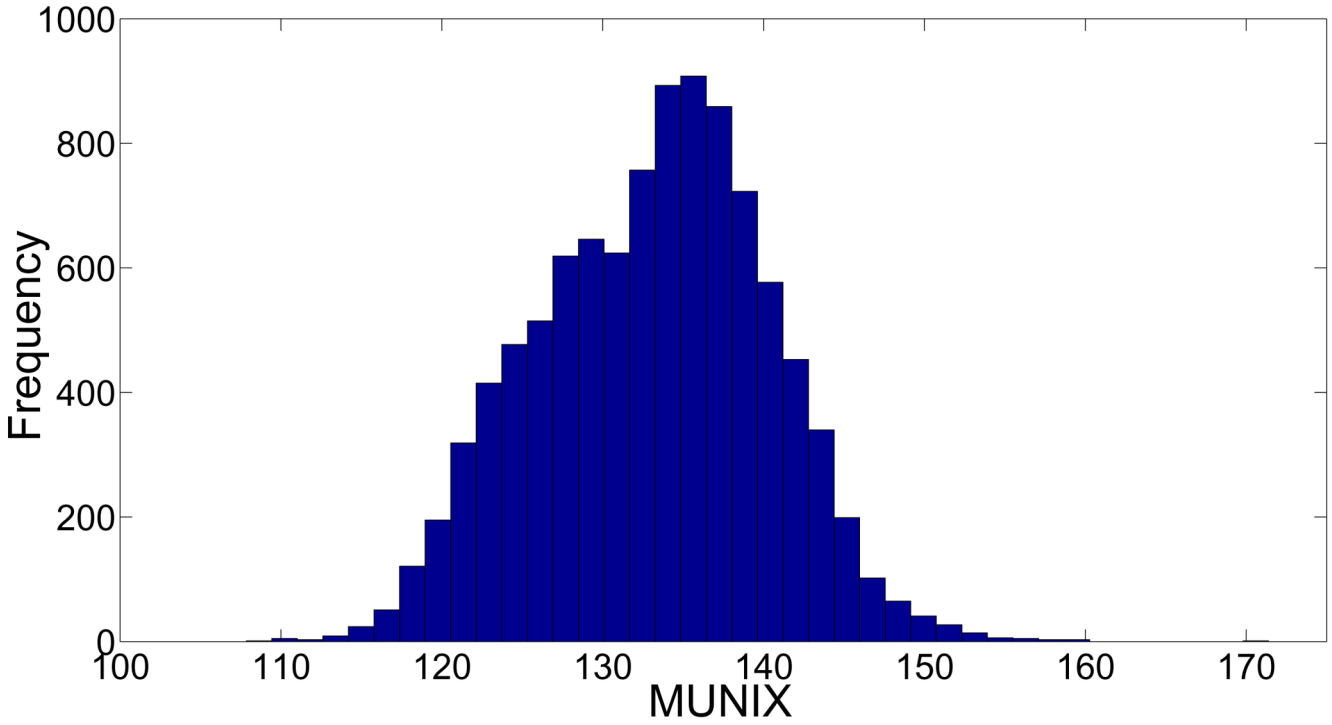

Figure 4.

Histogram of MUNIX measurements (N= 10000) for subject # 1 under the original MUNIX setting ($N_5 = 0$). A well-formed bell-shape normal distribution was observed as well as in other subjects of other epoch combinations.

Table 1

Summary of original MUNIX results (each contraction only has one epoch).

Sub #	Mean	STD	5%	95%	Range
1	129.8	5.8	119.4	136.4	17.1
2	84.1	1.8	82.0	86.4	4.4
3	115.8	3.1	109.8	119.9	10.1
4	103.1	2.7	100.0	106.9	6.9
5	107.9	0.8	106.3	109.0	2.7
6	126.0	3.9	121.4	131.4	10.0
7	128.1	4.2	124.5	134.7	10.2
8	152.3	7.8	143.1	167.3	24.1
Mean	118.4	3.8	113.3	124.0	10.7
STD	20.6	2.2	18.3	24.3	7.0

Table 2

Summary of results using the original MUNIX and the first strategy (Five contraction levels, N_5 epochs out of 10 randomly selected in each contraction).

	Original MUNIX N = 10 (N_5 = 0)			**Solution – 1 N = 16 (N_5 = 1)**			**Solution – 1 N = 22 (N_5 = 2)**			**Solution – 1 N = 28 (N_5 = 3)**			**Solution – 1 N = 34 (N_5 = 4)**		
Sub #	**Mean**	**STD**	**Range**	**Mean**	**STD**	**Range**	**Mean**	**STD**	**Range**	**Mean**	**STD**	**Range**	**Mean**	**STD**	**Range**
1	133.1	7.2	23.1	133.1	5.7	18.3	132.9	4.9	15.9	133.0	4.4	14.3	133.0	3.9	12.9
2	84.3	5.2	15.6	84.1	3.2	10.3	84.1	2.5	8.1	84.2	2.1	6.8	84.1	1.8	6.1
3	113.1	7.8	25.5	112.7	6.6	21.2	112.2	6.1	18.9	112.0	5.6	16.9	111.7	5.1	15.3
4	105.1	3.7	12.4	105.4	2.8	9.2	105.4	2.4	7.8	105.5	2.1	6.9	105.5	1.9	6.2
5	106.7	7.2	24.8	106.2	5.7	19.0	105.9	4.7	15.2	105.6	3.9	12.6	105.5	3.3	11.0
6	124.3	9.5	28.2	124.0	6.0	19.9	123.7	4.5	14.9	123.6	3.6	11.8	123.6	3.0	10.0
7	120.0	7.4	25.2	119.0	5.2	17.1	118.8	4.1	13.3	118.7	3.4	11.3	118.5	2.9	9.5
8	153.5	12.9	41.3	152.5	9.9	30.0	151.8	8.8	26.4	151.0	8.2	23.9	150.2	7.8	22.1
Mean	117.5	7.6	24.5	117.1	5.6	18.1	116.9	4.7	15.1	116.7	4.2	13.1	116.5	3.7	11.6
STD	20.6	2.7	8.7	20.4	2.2	6.5	20.3	2.0	6.0	20.1	2.0	5.6	19.9	2.0	5.2
p				0.0336	0.0004	0.0002	0.0208	0.0003	0.0001	0.0338	0.0002	0.0001	0.0419	0.0001	0.0001

Notes:

1. Ranges were calculated as the difference between 5% and 95% percentile MUNIX values (10,000 measurements).
2. Statistical analyses were compared with the original MUNIX using paired two-sided t-test.

Table 3

Summary of results using additional contraction levels and combined solutions (Nine contraction levels, N_9 epochs were randomly selected out of 10 in each contraction).

	Solution – 2 N = 18 (N_9 = 0)			**Solution – 1,2 N = 28 (N_9 = 1)**			**Solution – 1,2 N = 38 (N_9 = 2)**			**Solution – 1,2 N = 48 (N_9 = 3)**			**Solution – 1,2 N = 58 (N_9 = 4)**		
Sub #	**Mean**	**STD**	**Range**	**Mean**	**STD**	**Range**	**Mean**	**STD**	**Range**	**Mean**	**STD**	**Range**	**Mean**	**STD**	**Range**
1	130.4	4.8	15.6	130.4	3.7	12.2	130.4	3.2	10.7	130.5	2.9	9.4	130.4	2.6	8.5
2	84.5	3.4	11.7	84.3	2.4	8.0	84.3	2.0	6.5	84.3	1.7	5.5	84.3	1.5	5.0
3	109.7	5.9	20.2	109.4	4.2	13.6	109.2	3.2	10.5	109.1	2.7	8.6	109.0	2.2	7.2
4	104.4	3.0	9.9	104.5	2.2	7.2	104.5	1.9	6.1	104.5	1.6	5.4	104.5	1.5	5.0
5	106.2	5.1	16.6	106.0	4.0	12.9	105.9	3.3	10.6	105.8	2.7	9.0	105.8	2.4	7.7
6	125.9	7.6	24.4	124.9	5.3	17.6	124.7	4.1	13.4	124.4	3.4	11.0	124.4	2.8	9.3
7	120.0	7.1	24.2	119.3	5.1	16.9	119.3	4.1	13.3	119.1	3.4	11.1	119.0	2.9	9.5
8	152.8	9.6	32.0	152.1	6.8	22.2	151.6	5.4	17.8	151.4	4.6	15.2	151.3	4.0	13.3
Mean	116.7	5.8	19.3	116.4	4.2	13.8	116.3	3.4	11.1	116.1	2.9	9.4	116.1	2.5	8.2
STD	20.5	2.2	7.3	20.3	1.5	5.0	20.2	1.2	3.8	20.1	1.0	3.2	20.1	0.8	2.7
p	0.2090	0.0008	0.0015	0.0576	0.0002	0.0002	0.0397	0.0002	0.0001	0.0264	0.0002	0.0001	0.0238	0.0002	0.0001

Notes:

1. Ranges were calculated as the difference between 5% and 95% percentile MUNIX values (10,000 measurements).
2. Statistical analyses were compared with the original MUNIX using paired two-sided t-test.