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## **Larger and denser: an optimal design for surface grids of EMG electrodes to identify greater and more representative samples of motor units**

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1        **1. Manuscript Title**

2        Larger and denser: an optimal design for surface grids of EMG electrodes to identify greater  
3        and more representative samples of motor units

4        **2. Abbreviated title**

5        HD-EMG decomposition with larger and denser grids

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37

38 **Abstract**

39 The spinal motor neurons are the only neural cells whose individual activity can be non-invasively  
40 identified. This is usually done using grids of surface electromyographic (EMG) electrodes and source  
41 separation algorithms; an approach called EMG decomposition. In this study, we combined  
42 computational and experimental analyses to assess how the design parameters of grids of electrodes  
43 influence the number and the properties of the identified motor units. We first computed the  
44 percentage of motor units that could be theoretically discriminated within a pool of 200 simulated  
45 motor units when decomposing EMG signals recorded with grids of various sizes and interelectrode  
46 distances (IED). Increasing the density, the number of electrodes, and the size of the grids, increased  
47 the number of motor units that our decomposition algorithm could theoretically discriminate, i.e., up to  
48 83.5% of the simulated pool (range across conditions: 30.5-83.5%). We then identified motor units  
49 from experimental EMG signals recorded in six participants with grids of various sizes (range: 2-36  
50 cm<sup>2</sup>) and IED (range: 4-16 mm). The configuration with the largest number of electrodes and the  
51 shortest IED maximized the number of identified motor units (56±14; range: 39-79) and the  
52 percentage of early recruited motor units within these samples (29±14%). Finally, the number of  
53 identified motor units further increased with a prototyped grid of 256 electrodes and an IED of 2 mm.  
54 Taken together, our results showed that larger and denser surface grids of electrodes allow to identify a  
55 more representative pool of motor units than currently reported in experimental studies.

56

57 **Significance statement**

58 The application of source separation methods to multi-channel EMG signals recorded with grids of  
59 electrodes enables users to accurately identify the activity of individual motor units. However, the  
60 design parameters of these grids have never been discussed. They are usually arbitrarily fixed, often  
61 based on commercial availability. Here, we showed that using larger and denser grids of electrodes  
62 than conventionally proposed drastically increases the number of identified motor units. The samples  
63 of identified units are more balanced between early- and late-recruited motor units. Thus, these grids  
64 provide a more representative sampling of the active motor unit population. Gathering large datasets  
65 of motor units using large and dense grids will impact the study of motor control, neuromuscular  
66 modelling, and human-machine interfacing.

67

68 **Introduction**

69 Decoding the neural control of natural behaviors relies on the identification of the discharge activity of  
70 individual neural cells. Classically, arrays of electrodes are implanted close to the cells to record their  
71 electrical activity. The application of algorithms that separate the simultaneous and overlapping  
72 activity of these cells has enabled researchers to study neural processes in multiple areas of the brain  
73 (Stringer et al., 2019), such as in the motor or the sensorimotor areas (Churchland and Shenoy, 2007;  
74 Gallego et al., 2020). At the periphery of the nervous system, it is also possible to record the activity  
75 of individual motor neurons innervating muscle fibers (Duchateau and Enoka, 2011; Heckman and  
76 Enoka, 2012; Farina et al., 2016). The motor unit, i.e., a motor neuron and the muscle fibers it  
77 innervates, acts as an amplifier of the neural activity, as one action potential propagating along a motor  
78 neuron's axon generates an action potential in each of the innervated muscle fibers. The discharge  
79 activity of motor units can be identified by decomposing surface electromyographic (EMG) signals  
80 into trains of motor unit action potentials (MUAPs) using, e.g., blind-source separation algorithms  
81 (Holobar and Farina, 2014; Farina and Holobar, 2016). The multiple observations for source  
82 separation are obtained by recording EMG signals with grids of electrodes. This approach usually  
83 allows for the reliable analysis of 5 to 40 concurrently active motor units (Del Vecchio et al., 2017;  
84 Del Vecchio et al., 2020; Hug et al., 2021a).

85 While the design of intracortical (e.g., (Jun et al., 2017; Steinmetz et al., 2018)) and intramuscular  
86 (e.g., (Muceli et al., 2015; Muceli et al., 2022)) electrodes arrays has scaled up over the years to record  
87 larger samples of neural cells, the configuration of grids of surface EMG electrodes has not  
88 systematically evolved. Most researchers currently use grids with 64 electrodes arranged in  $13 \times 5$  or  $8$   
89  $\times 8$  montages, the interelectrode distance (IED) between adjacent electrodes (e.g., 4 mm, 8 mm, or 10  
90 mm) being dictated by the size of the muscle to cover. Yet, optimizing these parameters, i.e., grid size  
91 and IED, may influence the performance of EMG decomposition. Currently, there are no  
92 recommendations on optimal designs for grids of electrodes.

93 Source separation algorithms are based on the necessary condition that identifiable motor units have a  
94 unique representation of their action potentials across the multi-channel EMG signals (Farina et al.,  
95 2008; Holobar and Farina, 2014; Farina and Holobar, 2016). This implies that the three-dimensional  
96 waveform of a MUAP (one time dimension and two spatial dimensions) is unique within the pool of  
97 active motor units detected by the grid of electrodes. In practice, the identified motor units are those  
98 that innervate larger numbers of muscle fibers, as their action potentials tend to have the largest  
99 energy. Conversely, low-threshold motor units usually remain hidden since their energy is close to the  
100 baseline noise. Increasing the density of electrodes would increase the spatial sampling of EMG  
101 signals (Farina and Holobar, 2016), which in turn should improve the discrimination of MUAPs,  
102 allowing the identification of a higher number of motor units. Additionally, increasing the density of

103 electrodes may reveal the hidden low-threshold motor units by sampling their action potentials across  
104 a higher number of electrodes, leading to a better compensation of the additive noise in the mixture  
105 model of the EMG signal (Farina and Holobar, 2016).

106 In this study, we combined computational and laboratory experiments to identify the optimal design  
107 parameters of grids of surface electrodes with the aim to maximize the number of identified motor  
108 units. We first simulated a pool of 200 motor units and the associated EMG signals recorded from  
109 grids of electrodes of various sizes and densities. These simulations showed that the greater the size  
110 and the density of the grid, the higher the percentage of theoretically identifiable motor units and the  
111 relative ratio of theoretically identifiable deep units. We confirmed these theoretical results with  
112 experimental signals recorded with a grid of 256 electrodes with a 4-mm IED that was down-sampled  
113 in the space domain to obtain six grid configurations (surface range: 2-36 cm<sup>2</sup> and IED range: 4-16  
114 mm). Finally, we prototyped a new grid of 256 electrodes with a 2-mm IED and demonstrated that the  
115 number of identified motor units further increased with 2-mm IED. The entire dataset (raw and  
116 processed data) and codes are available at <https://figshare.com/s/f4a94d9bdf470bf10f8>.

117 **Methods**

118 **Computational study**

119 A pool of 200 motor units was simulated to test whether increasing the density and the size of surface  
120 grids of electrodes would impact the number of theoretically identifiable motor units. The simulations  
121 were based on an anatomical model entailing a cylindrical muscle volume with parallel fibers (Farina  
122 et al., 2008; Konstantin et al., 2020), in which subcutaneous and skin layers separate the muscle from  
123 the surface electrodes. Specifically, we set the radius of the muscle to 25.4 mm and the thicknesses of  
124 the subcutaneous and skin layers to 5 mm and 1 mm, respectively. The centers of the motor units were  
125 distributed within the cross section of the muscle using a farthest point sampling technique. The  
126 farthest point sampling filled the cross-section by iteratively adding centers points that were  
127 maximally distant from all the previously generated motor unit centers, resulting in a random and even  
128 distribution of the motor unit territories within the muscle. The number of fibers innervated by each  
129 motor neuron followed an exponential distribution, ranging from 15 to 1500. The fibers of the same  
130 motor unit were positioned around the center of the motor unit within a radius of 0.2 to 9.8 mm, and a  
131 density of 20 fibers/mm<sup>2</sup>. Because motor unit territories were intermingled, the density of fibers in the  
132 muscle reached 200 fibers/mm<sup>2</sup>. The MUAPs were detected by circular surface electrodes with a  
133 diameter of 1 mm. The simulated grids were centered over the muscle in the transverse direction, with  
134 a size ranging from 14.4 to 36 cm<sup>2</sup>, and an IED ranging from 2 to 36 mm.

135

136 **Laboratory study**

137 Participants

138 Six healthy participants (all males; age: 26 ± 4 yr; height: 174 ± 7 cm; body weight: 66 ± 15 kg)  
139 volunteered to participate in the first experimental session of the study. They had no history of lower  
140 limb injury or pain during the months preceding the experiments. One of these individuals (age: 26 yr;  
141 height: 168 cm; bodyweight: 51 kg) participated in a second experimental session to test the  
142 prototyped grid with an IED of 2 mm. The Ethics Committee at Imperial College London reviewed  
143 and approved all procedures and protocols (no. 18IC4685). All participants provided their written  
144 informed consent before the beginning of the experiment.

145

146 Experimental tasks

147 The two experimental sessions consisted of a series of isometric ankle dorsiflexions performed at 30%  
148 and 50% of the maximal voluntary torque (MVC) during which we recorded high density  
149 electromyographic (HD-EMG) signals over the Tibialis Anterior muscle (TA). The participant sat on a  
150 massage table with the hips flexed at 30°, 0° being the hip neutral position, and their knees fully  
151 extended. We fixed the foot of the dominant leg (right in all participants) onto the pedal of a

152 commercial dynamometer (OT Bioelettronica, Turin, Italy) positioned at 30° in the plantarflexion  
153 direction, 0° being the foot perpendicular to the shank. The thigh was fixed to the massage table with  
154 an inextensible 3-cm-wide Velcro strap. The foot was fixed to the pedal with inextensible straps  
155 positioned around the proximal phalanx, metatarsal and cuneiform. Force signals were recorded with a  
156 load cell (CCT Transducer s.a.s, Turin, Italy) connected in-series to the pedal using the same  
157 acquisition system as for the HD-EMG recordings (EMG-Quattrocento; OT Bioelettronica). The  
158 dynamometer was positioned accordingly to the participant's lower limb length and secured to the  
159 massage table to avoid any motion during the contractions.

160 All experiments began with a warm-up, consisting of brief and sustained ankle dorsiflexion performed  
161 at 50% to 80% of the participant's subjective MVC. During the warm-up, all participants learnt to  
162 produce isometric ankle dorsiflexion without co-contracting the other muscles crossing the hip and  
163 knee joints. At the same time, we iteratively adjusted the tightening and the position of the straps to  
164 maximize the comfort of the participant. Then, each participant performed two 3-to-5 s MVC with 120  
165 s of rest in between. The peak force value was calculated using a 250-ms moving average window, and  
166 then used to set the target level during the submaximal contractions. After 120 s of rest, each  
167 participant performed two trapezoidal contractions at 30% and 50% MVC with 120 s of rest in  
168 between, consisting of linear ramps up and down performed at 5%/s and a plateau maintained for 20 s  
169 and 15 s at 30% and 50% MVC, respectively. The order of the contractions was randomized. One  
170 participant (S2) did not perform the contractions at 50% MVC.

171

#### 172 High-density electromyography

173 In the first experimental session, four adhesive grids of 64 electrodes (13 x 5 with a missing electrode  
174 in a corner; gold coated; 1 mm diameter; 4 mm IED; OT Bioelettronica) were placed over the belly of  
175 the TA. The grids were carefully positioned side-to-side with a 4-mm-distance between the electrodes  
176 at the edges of adjacent grids (Figure 1A). The 256 electrodes were centered to the muscle belly and  
177 laid within the muscle perimeter identified through palpation. The skin was shaved, abraded and  
178 cleansed with 70% ethyl alcohol. Electrode-to-skin contact was maintained with a bi-adhesive  
179 perforated foam filled with conductive paste. The grids were wrapped with tape and elastic bands to  
180 secure the contact with the skin. The four pre-amplifiers were connected in-series with stackable  
181 cables to a wet reference band placed above the medial malleolus of the same leg. HD-EMG signals  
182 were recorded in monopolar derivation with a sampling frequency of 2,048 Hz, amplified (x150),  
183 band-pass filtered (10–500 Hz), and digitized using a 400 channels acquisition system with a 16-bit  
184 resolution (EMG-Quattrocento; OT Bioelettronica).

185 In the second experimental session, one ultra-dense prototyped grid of 256 electrodes (Figure 1H; 26 x  
186 10 with a missing electrode in each corner; gold coated; 1 mm diameter; 9 cm<sup>2</sup> area; 2-mm IED;

187 custom-manufactured for this study by OT Bioelettronica) was placed over the belly of the TA and the  
188 HD-EMG signals were recorded using the same procedure as previously described.

189

#### 190 Grid configurations

191 During the first experimental session, we recorded EMG signals from the TA with a total of 256  
192 electrodes covering an area of 36 cm<sup>2</sup> over the muscle (10 cm x 3.6 cm, 4-mm IED, Figure 1A). To  
193 investigate the effect of electrode density, we down-sampled the grid of 256 electrodes by successively  
194 discarding rows and columns of electrodes and artificially generating three new grids covering the  
195 same area with IEDs of 8 mm, 12 mm, and 16 mm, that involved 256, 64, 35, and 20 electrodes,  
196 respectively (Figure 1B-D). It is noteworthy that the 8-mm and 16-mm grids covered a surface of 32  
197 cm<sup>2</sup> because they included an odd number of rows and columns. To investigate the effect of the size of  
198 the grid, we discarded the peripheral electrodes to generate grids of 63, 34 and 19 electrodes with a  
199 4-mm IED, covering areas of 7.7, 3.8 and 2 cm<sup>2</sup> over the muscle (Figure 1E-G). We chose these sizes  
200 to match the number of electrodes used in the density analysis, thus comparing grids with similar  
201 number of electrodes, but different densities and sizes (in Figure 1, B versus E, and C versus F).

202 During the second experimental session, we recorded EMG signals from the TA with an ultra-dense  
203 grid of 256 electrodes covering an area of 9 cm<sup>2</sup> over the muscle (5 cm x 1.8 cm, 2-mm IED, Figure  
204 1H). Using the same procedure as above, we generated two artificial grids of 64 and 32 electrodes with  
205 an IED of 4 mm and 8 mm, respectively.

206

#### 207 HD-EMG decomposition

208 We decomposed the signals recorded in all the conditions using the same algorithm, parameters, and  
209 procedure. First, the monopolar EMG signals were band-pass filtered between 20 and 500 Hz with a  
210 second-order Butterworth filter. The channels with low signal-to-noise ratio or artifacts were discarded  
211 after visual inspection. The HD-EMG signals were then decomposed into individual motor unit pulse  
212 trains using convolutive blind-source separation, as previously described (Negro et al., 2016). In short,  
213 the EMG signals were first extended by adding delayed versions of each channel. We kept the same  
214 extension factor for all the conditions to reach 1000 extended channels, as previously suggested  
215 (Negro et al., 2016). The extended signals were spatially whitened to make them uncorrelated and of  
216 equal power. Thereafter, a fixed-point algorithm was applied to identify the sources embedded in the  
217 EMG signals, i.e., the motor unit pulse trains, or series of delta functions centered at the motor unit  
218 discharge times. In this algorithm, the contrast function  $g(x) = \log(\cosh(x))$  was iteratively applied to  
219 the EMG signals to skew the distribution of the values of the motor unit pulse trains toward 0, and thus  
220 maximize the level of sparsity of the motor unit pulse train. The high level of sparsity matches the



221 physiological properties of motor units, with a relatively small number of discharges per second (< 50  
222 discharge times/s during submaximal isometric contractions). The convergence was reached once the  
223 level of sparsity did not substantially vary (with a tolerance fixed at  $10^{-4}$ ) when compared to the  
224 previous iteration (Negro et al., 2016). At this stage, the motor unit pulse train contained high peaks  
225 (i.e., the delta functions from the identified motor unit) and lower values due to the activities of other  
226 motor units and noise. High peaks were separated from lower values using peak detection and K-mean  
227 classification with two classes. The peaks from the class with the highest centroid were considered as  
228 the discharge times of the identified motor unit. A second algorithm refined the estimation of the  
229 discharge times by iteratively recalculating the motor unit filter and repeating the steps with peak  
230 detection and K-mean classification until the coefficient of variation of the inter-spike intervals was  
231 minimized. This decomposition procedure has been previously validated using experimental and  
232 simulated signals (Negro et al., 2016). After the automatic identification of the motor units, duplicates  
233 were automatically removed. For this purpose, the pulse trains identified from pairs of motor units  
234 were first aligned using a cross-correlation function to account for a potential delay due to the  
235 propagation time of action potentials along the fibers. Then, two discharge times were considered as  
236 common when they occurred within a time interval of 0.5 ms, and two or more motor units were  
237 considered as duplicates when they had at least 30% of their identified discharge times in common  
238 (Holobar et al., 2010). In principle, the limited level of synchronization between individual motor units  
239 results in a few simultaneous discharges between pairs of motor units. A threshold of 30% is therefore  
240 highly conservative to ensure the removal of all motor units with a level of synchronization well above  
241 physiological values. It is worth noting that most of the motor units identified as duplicates after the  
242 automatic decomposition had almost 100% of their discharge times in common. In that case, the motor  
243 unit with the lowest coefficient of variation of the inter-spike intervals was retained for the analyses.  
244 At the end of these automatic steps, all the motor unit pulse trains, i.e., the output of the decomposition  
245 resulting from the projection of EMG signals onto individual motor unit filters, were visually  
246 inspected, and manual editing was performed to correct the false identification of artifacts or the  
247 missed discharge times (Del Vecchio et al., 2020; Hug et al., 2021b; Avrillon et al., 2023). The update  
248 of the motor unit filters with the corrected discharge times and the recalculation of the motor unit  
249 pulse trains always improved the distance between the discharge times and the noise, quantified with  
250 the pulse-to-noise ratio (PNR) (Holobar et al., 2014). Note that this manual step is highly reliable  
251 across operators, as previously demonstrated by Hug et al. (2021b). Duplicates were checked a second  
252 time after manual editing, with very rare cases of removal as most of the duplicates were automatically  
253 identified after the automatic decomposition. Only the motor unit pulse trains which exhibited a PNR  
254 > 28 dB after manual editing were retained for further analysis.

255

256 We further tested whether decomposing subsets of electrodes within a highly populated grid of 256  
257 electrodes increased the number of identified motor units. Indeed, the lower ratio of large motor units  
258 sampled by each independent subset of 64 electrodes could allow the algorithm to converge to smaller  
259 motor units that contribute to the signal. For a similar number of iterations, it is likely that these motor  
260 units would have otherwise contributed to the noise component of the mixture model of the EMG  
261 signal (Farina and Holobar, 2016). Thus, we decomposed the grids of 256 electrodes (4-mm and 2-mm  
262 IED, Figure 1A, H) as four separated grids of 64 electrodes before removing the motor units  
263 duplicated between grids.

264

265

## 266 **Analyses**

### 267 Computational study

268 We first estimated the theoretical percentage of identifiable motor units for each of the simulated  
269 conditions. To do so, the simulated MUAPs detected over the entire set of electrodes were compared  
270 with each other. The comparisons were done pairwise by first aligning the MUAPs in time using the  
271 cross-correlation function, and then computing the normalized mean square difference between the  
272 aligned action potentials. Pairs of action potentials with a mean square difference below 5% were  
273 considered not discriminable. The 5% criterion was based on the variability of motor unit action  
274 potential shapes observed experimentally for individual motor units (Farina et al., 2008). After  
275 computing all pair-wise comparisons, we then computed the percentage of action potentials that could  
276 be discriminated from all others, i.e., the theoretical percentage of identifiable motor units. This metric  
277 is independent from the algorithm used for decomposition and establishes a theoretical upper bound in  
278 the number of motor units that can be identified by any decomposition algorithm. For each  
279 theoretically identifiable motor unit, we also computed the distance between the center of the territory  
280 of the corresponding muscle fibers and the skin surface.

281

### 282 Laboratory study – number of identified motor units

283 We reported the absolute number of motor units (PNR > 28 dB) identified with all the grid  
284 configurations. For each participant, the number of identified motor units was then normalized to the  
285 maximal number of motor units found across all conditions, yielding normalized numbers of identified  
286 motor units  $\bar{N}$  expressed in percentage. For each condition, we calculated the mean and standard  
287 deviation of the  $\bar{N}$  values across participants. To investigate the effects of density and size of the grid,  
288 we fitted logarithmic trendlines to the relationships between the averaged  $\bar{N}$  values and IED or grid

289 size. We also fitted a logarithmic trendline to the average  $\bar{N}$  values and their corresponding number of  
290 electrodes, in which case the conditions involving the same number of electrodes, but different grid  
291 size and density, were given a weight of 0.5 in the minimization function. We reported the  $r^2$  and p-  
292 value for each regression trendline. To maintain consistency with the computational study  
293 investigating the number of theoretically identifiable motor units across grid designs, the trendlines  
294 were fitted on the results obtained when the complete grids of 256 electrodes were decomposed as  
295 independent subsets of 64 electrodes, which systematically returned the highest number of identified  
296 motor units. The trendlines fitted on the results obtained with the decomposition of the 256 electrodes  
297 as a whole are reported in Figure 4-1.

298

#### 299 Laboratory study – properties of identified motor units

300 To investigate the effects of electrode density and grid size on the properties of the identified motor  
301 unit, we used a typical frequency distribution of the motor unit force recruitment thresholds in the  
302 human TA (Caillet et al., 2022b), where  $F^{th}(j)$  is the force recruitment threshold of the  $j^{\text{th}}$  motor unit  
303 in the normalized motor unit pool ranked in ascending order of  $F^{th}$ .

$$F^{th}(j) = 0.50 \cdot (58.12 \cdot j + 120j^{1.83}), j \in [0; 1]$$

304 The identified motor units were then classified according to this relationship and their measured force  
305 recruitment threshold, the first half of the active pool being ‘early recruited’, and the second half ‘late  
306 recruited’ (Henneman and Mendell, 1981; Caillet et al., 2022a). For each condition, we reported the  
307 percentage of identified motor units that were ‘early recruited’. We did not report this metric when  
308 five or fewer motor units were identified in one condition for three or more participants.

309

#### 310 Laboratory study – correlation between observations

311 We assessed how the density of electrodes impacted the information redundancy in EMG signals  
312 recorded by adjacent electrodes. To this end, MUAP shapes were identified over the 256 electrodes  
313 with the spike-triggered averaging technique. To do so, the discharge times were used as a trigger to  
314 segment and average the HD-EMG signals over a window of 50 ms. For each motor unit, we  
315 identified the electrode with the highest action potential peak-to-peak amplitude and calculated the  
316 average correlation coefficient  $\rho$  between this action potential and those recorded by the four adjacent  
317 electrodes with an IED of 4 mm, 8 mm, 12 mm, and 16 mm. We also repeated this correlation analysis  
318 for the ultra-dense grid of 256 electrodes using an IED of 2 mm, 4 mm, and 8 mm.

319 **Results**

320 All the datasets (raw and processed data) and codes used to process the data are available at  
321 <https://figshare.com/s/f4a94d9bdf470bf10f8>.

322

323 **Computational study**

324 We simulated the discharge activity of 200 motor units recorded by 84 configurations of grids of  
325 electrodes (Figure 2; surface range: 14.4 to 36 cm<sup>2</sup>, IED range: 2 to 36 mm). The number of  
326 theoretically identifiable motor units increased with the size of the grid, from 46.7 ± 7.7% of the motor  
327 units theoretically identifiable with a grid of 14.4 cm<sup>2</sup> to 77.8 ± 5.5% of the motor units theoretically  
328 identifiable with a grid of 36 cm<sup>2</sup>. The number of theoretically identifiable motor units also increased  
329 with shorter interelectrode distances. For example, with a grid of 36 cm<sup>2</sup>, the number of theoretically  
330 identifiable motor units increased from 63.5% to 83.5% of the motor units with an IED of 36 and 2  
331 mm, respectively (Figure 2B). Increasing the surface size and the density of the grid of electrodes  
332 revealed deeper motor units. The averaged distance of theoretically identifiable motor units from the  
333 skin increased with the size of the grid (Figure 2C; 14.3 ± 0.1 mm vs. 16.5 ± 0.2 mm with grids of  
334 14.4 and 36 mm<sup>2</sup>, respectively), but not with the IED of the grid (Figure 2D; 15.6 ± 1.1 mm vs. 15.5 ±  
335 0.9 mm with an IED of 36 and 2 mm, respectively).

336

337 **Laboratory study - grids of 256 electrodes with an IED of 4-mm**

338 Number of identified motor units

339 The motor unit pulse trains automatically identified across all conditions, intensities, and participants  
340 were visually inspected and carefully edited when a missing discharge time or a falsely identified  
341 artifact were observed. On average, 9 ± 4 % and 22 ± 9 % of the motor units automatically identified  
342 at 30% and 50% MVC, respectively, were removed after visual inspection and manual editing.  
343 Furthermore, when the four grids of 64 electrodes were separately decomposed, 30 ± 5 % and 24 ± 6  
344 % of the automatically identified motor units were removed because they were identified in more than  
345 one grid (only one pulse train was retained in case of duplicates). The highest number of identified  
346 motor units was systematically reached with the separate decomposition of the four grids of 64  
347 electrodes with an IED of 4 mm, with 56 ± 14 motor units (PNR = 34.2 ± 1.1) and 45 ± 10 motor units  
348 (PNR = 34.0 ± 0.9) at 30% and 50% MVC, respectively (Figure 3). At least 82% of the motor units  
349 identified in one condition were also identified in the conditions involving a higher number of  
350 electrodes. Similarly, 91% to 100% of the motor units identified in one condition were also identified  
351 with the 256-electrode configuration (4-mm IED, 36-cm<sup>2</sup> size, Figure 1A) with the four grids  
352 decomposed separately.

353

354 When considering the effect of electrode density (grid size fixed at 32-36 cm<sup>2</sup>, Figure 1A-D), we  
355 found the lowest number  $N$  of identified motor units with the 16-mm IED, with  $3 \pm 1$  motor units and  
356  $2 \pm 1$  motor units at 30% and 50% MVC, respectively (Figure 4A, C). Additional motor units were  
357 gradually identified with greater electrode densities. The highest number of identified motor units was  
358 observed with the highest density (4-mm IED), with  $56 \pm 14$  and  $45 \pm 10$  motor units at 30% and 50%  
359 MVC, respectively, with the 4×64-electrode decomposition procedure (Figure 4A, C). With the 256-  
360 electrode decomposition procedure,  $43 \pm 11$  and  $25 \pm 6$  motor units were identified at 30% and 50%  
361 MVC, respectively (Figure 4A, C). Finally, we found a decreasing logarithmic relationship between  
362 the normalized number  $\bar{N}$  of motor units, averaged for each participant, and the IED, with  $r^2 = 1.0$  ( $p =$   
363  $2.5 \cdot 10^{-5}$ ) and  $r^2 = 0.99$  ( $p = 0.001$ ) at 30% and 50% MVC, respectively (Figure 4B, D).

364 When considering the effect of the size of the grid (IED fixed at 4 mm, Figure 1A, E-G), we found the  
365 lowest number  $N$  of motor units with a grid of 2 cm<sup>2</sup>, with  $4 \pm 2$  motor units and  $4 \pm 2$  motor units at  
366 30% and 50% MVC, respectively (Figure 5A, C). Additional motor units were then gradually  
367 identified with larger grid sizes. The highest number of motor units was observed with a grid of 36  
368 cm<sup>2</sup>, with  $56 \pm 14$  and  $45 \pm 10$  motor units at 30% and 50% MVC, respectively, with the 4×64-  
369 electrode decomposition procedure (Figure 4A, C). With the 256- electrode decomposition procedure,  
370  $43 \pm 11$  and  $25 \pm 6$  motor units were identified at 30% and 50% MVC, respectively (Figure 4A, C).  
371 Finally, we found an increasing logarithmic relationship between the normalized number of motor  
372 units  $\bar{N}$ , averaged for each participant, and the size of the grid, with  $r^2 = 0.99$  ( $p = 3.0 \cdot 10^{-4}$ ) and  $r^2 =$   
373  $0.98$  ( $p = 0.001$ ) at 30% and 50% MVC, respectively (Figure 5B, D). It is noteworthy that the  
374 parameters of the fits were very similar at 30% and 50% MVC in both analyses.

375 As both the density and the size of the grid determine the number of electrodes, we finally fitted the  
376 relationship between the normalized number of motor units  $\bar{N}$  and the number of electrodes. As  
377 observed previously, more motor units were identified with a larger number of electrodes, following a  
378 logarithmic tendency with  $r^2 = 0.98$  ( $p = 0.018$ ) and  $r^2 = 0.95$  ( $p = 0.016$ ) at 30% and 50% MVC,  
379 respectively (Figure 6). A plateau should theoretically be reached with grids of 1024 and 4096  
380 electrodes (36-cm<sup>2</sup> grids with 2-mm and 1-mm IED, respectively), with a prediction of 50% and 90%  
381 more motor units.

382 For a fixed number of electrodes, it is noteworthy that the size and the density, although linked, may  
383 have different impact on the number of identified motor units (black crosses in Figure 6). For example,  
384 1.25 times more motor units were obtained with the 64-electrode condition (32 cm<sup>2</sup>, 8-mm IED,  
385 Figure 1B) than with the 63-electrode condition (7.7 cm<sup>2</sup>, 4-mm IED, Figure 1E) for the group of  
386 participants at 30% MVC.

387

388 Characteristics of identified motor units

389 We found an increasing logarithmic relationship between the percentage of early recruited motor units  
390 for each participant and the density of the grid, with  $r^2 = 0.91$  ( $p = 2.8 \cdot 10^{-3}$ ) at 30% MVC (Figure 7F).  
391 Contrary to the density, the size of the grid did not impact the percentage of early recruited motor  
392 units, with the percentage ranging from 20 to 29% across all sizes, and the logarithmic trendline  
393 returning a negligible slope and a low  $r^2 = 0.28$  (Figure 7C, G). Such differences were also not  
394 observed at 50% MVC, where the percentage of early recruited motor units remained below 10% for  
395 all conditions.

396 To support the above observations made at 30% MVC, grids with the same number of electrodes, but  
397 different densities and sizes, were directly compared. 62% of the motor units identified with the grids  
398 of 64 electrodes (32 cm<sup>2</sup>, IED 8 mm) and 63 electrodes (7.7 cm<sup>2</sup>, IED 4 mm) were identified in both  
399 conditions at 30% MVC.  $28 \pm 9\%$  of the motor units specific to the 8-mm IED grid were early  
400 recruited, while  $44 \pm 11\%$  of the motor units specific to the 4-mm IED condition were early recruited.  
401 Similar results were obtained with the grids of 35 (36 cm<sup>2</sup>, 12-mm IED) and 34 electrodes (3.6 cm<sup>2</sup>, 4-  
402 mm IED), where a higher number of early recruited motor units were specifically identified with  
403 denser rather than larger grids.

404

405 Correlation between MUAPs from adjacent electrodes

406 Figure 8 reports the effect of the density of electrodes on the level of correlation  $\rho$  between the profiles  
407 of action potentials recorded by adjacent electrodes. The lowest average correlation coefficient  $\rho$  was  
408 observed with an IED of 16 mm ( $\rho = 0.87 \pm 0.03$  and  $\rho = 0.88 \pm 0.04$  at 30% and 50% MVC,  
409 respectively). The level of correlation increased with a shorter IED, with  $\rho = 0.96 \pm 0.04$  and  $\rho = 0.95$   
410  $\pm 0.05$  between the profiles of action potentials recorded by adjacent electrodes with a 4-mm IED at  
411 30% and 50% MVC, respectively (Figure 8B, C).

412

413 **Laboratory study with an ultra-dense prototyped grid of 256 electrodes with 2-mm IED**

414 31 and 26 motor units (PNR > 28 dB) were identified for one participant with the ultra-dense grid of  
415 256 electrodes (2-mm IED, 9 cm<sup>2</sup>, Figure 1H) at 30% and 50% MVC, respectively (Figure 9B). Note  
416 that the signals from four independent subsets of 64 electrodes were decomposed separately. For that  
417 participant, more motor units were identified with the ultra-dense grid of 256 electrodes than with the  
418 grid of 64 electrodes covering the same area (Figure 5A, C). Indeed, 31 and 26 motor units were  
419 respectively identified at 30% and 50% MVC with the grid of 256 electrodes (Figure 9C), while 25

420 (24 ± 5 for the group) and 19 (18 ± 4 for the group) motor units were identified with the grid of 64  
421 electrodes (Figure 5A, C). Moreover, fewer motor units were identified when the electrode density of  
422 the ultra-dense grid was decreased (Figure 9C), with 22 and 13 motor units identified with a 4- and 8-  
423 mm IED at 30% MVC, respectively, and 21 and 9 motor units identified with a 4- and 8-mm IED at  
424 50% MVC, respectively. At 30% MVC, the rate of increase of N between 4- and 2-mm IED followed  
425 the prediction computed in Figure 4B and illustrated by the dash line in Figure 9C. At 50% MVC, the  
426 rate of increase of N (dotted line in Figure 9C) was lower than the prediction. As previously observed,  
427 the correlation between adjacent MUAPs increased from  $\rho = 0.92$  with an 8-mm IED to  $\rho = 0.98$  with  
428 a 2-mm IED at 30% MVC, and from  $\rho = 0.85$  with an 8-mm IED to  $\rho = 0.93$  with a 2-mm IED at 50%  
429 MVC (Figure 9A). All the motor units identified with the 8-mm and 4-mm IED were also identified  
430 with the 4-mm and 2-mm IED grids, respectively. Finally, more motor units with an early recruitment  
431 were identified when increasing the density from 8- to 4-mm IED (blue vs black trains in Figure 9B),  
432 and from 4- to 2-mm IED (red trains in Figure 9B).

433

434 **Discussion**

435 This study systematically investigated how the design parameters of grids of surface EMG electrodes  
436 (grid size and electrode density) impact the number and the properties of the motor units identified  
437 with EMG decomposition. Using a combination of computational and experimental analyses, we  
438 found that larger and denser grids of electrodes than conventionally used reveal a larger sample of  
439 identified motor units. As most of the motor units that were not identified with less dense and smaller  
440 grids had an early recruitment threshold, we concluded that denser grids allow to identify smaller  
441 motor units. This is due to a better spatial sampling of MUAPs over the grid, which in turn improves  
442 the discrimination of motor units with a unique set of MUAPs among active motor units. These results  
443 clarify the direction for designing new grids of electrodes that could span across the entire surface of  
444 the muscle of interest while keeping a high density of electrodes, with IED as low as 2 mm.  
445 Identifying large sets of small and large motor units is relevant in many research areas related to motor  
446 control, such as the investigation of synergies (Hug et al., 2022), neuromuscular modelling (Caillet et  
447 al., 2022c), or human-machine interfacing (Farina et al., 2021).

448

449 The number  $N$  of identified motor units increased across participants with the density of electrodes  
450 (Figure 4; Figure 8C), the size of the grid (Figure 5), and the number of electrodes (Figure 6). On  
451 average, 30 and 19 motor units were identified with the ‘conventional’ 64-electrode grid (8-mm IED,  
452 32 cm<sup>2</sup> surface area) at 30% and 50% MVC, respectively, which is consistent with several previous  
453 studies using similar grid designs (Del Vecchio et al., 2020). By increasing the density of electrodes  
454 and size of the grid to reach a total of 256 electrodes separated by a 4-mm IED, we identified on  
455 average 56 and 45 motor units at 30% and 50% MVC, respectively. We even reached 79 and 59 motor  
456 units for one subject (Figure 3), which is substantially more than the numbers of motor units usually  
457 reported in studies with similar methods, and twice those obtained with grids of 64 electrodes in this  
458 study. Our computational and experimental analyses showed that the size of the grid is a key factor  
459 contributing to the higher number of identified motor units (Figure 2B; Figure 5). According to our  
460 simulations, increasing the size of the grid increases the number of theoretically identifiable motor  
461 units, i.e., the number of motor units with unique sets of MUAPs across electrodes (Figure 2B). These  
462 differences between MUAPs result from the anatomical and physiological differences between  
463 adjacent motor units, such as the length of their fibers, the spread of the end plates, or their conduction  
464 velocity, as well as from the properties of the tissues separating the fibers from each recording  
465 electrode (Farina et al., 2004). Larger grids better sample these differences across electrodes, revealing  
466 the unique profiles of each motor unit action potentials (Farina et al., 2008). The density of electrodes  
467 was also a critical factor to increase the number of identified motor units (Figure 4; Figure 9C). Dense  
468 grids especially allowed to better identify early recruited motor units. Classically, the decomposition



469 algorithms tend to converge towards the large and superficial motor units that contribute to most of the  
470 energy of the EMG signals (Farina and Holobar, 2016). Conversely, action potentials of the smallest  
471 motor units tend to have lower energy and are masked by the potentials of the larger units. These  
472 factors explain the lowest representation of low-threshold motor units in available HD-EMG datasets  
473 (Cailliet et al., 2023). Increasing the density of electrodes would therefore enable to better sample the  
474 action potential profiles of these early recruited motor units across multiple electrodes, enabling their  
475 identification. However, we observed that increasing the density did not reveal additional early  
476 recruited motor units during contractions at 50% MVC (Figure 7D). This is potentially due to the  
477 higher energy of the MUAPs of the motor units recruited between 30% and 50% MVC. Additionally,  
478 we also showed in one subject that synthetically increasing the density of electrodes by resampling  
479 EMG signals with spatial interpolation does not have the same effect as with denser grids. In this  
480 example, 4 and 19 motor units were identified from the interpolated grid with a 4-mm and 2-mm IED,  
481 respectively, vs. 19 and 24 motor units with the experimentally recorded signals. All the motor units  
482 identified with the interpolated grid were also identified with the experimentally recorded signals  
483 (Figure 4-2).

484

485 The number of identified motor units  $N$  monotonically increased with the density of electrodes (Figure  
486 4BD), the size of the grid (Figure 5BD) and the number of electrodes (Figure 6), following significant  
487 logarithmic trendlines. Remarkably, very similar logarithmic tendencies were obtained at both 30%  
488 and 50% MVC in all the analyses. Altogether, these trendlines suggested that the normalized number  
489 of identified motor units  $\bar{N}$  would grow with an electrode density beyond a 4-mm IED. We  
490 experimentally tested this hypothesis by designing a new prototyped grid of 256 electrodes separated  
491 by an IED of 2 mm. As predicted, more motor units were identified with a 2 mm than with a 4 mm  
492 IED, following at 30% MVC the same rate of increase as predicted by the logarithmic trendlines  
493 (Figure 9C) between 4-mm and 2-mm IED. This increase may plateau with higher electrode densities,  
494 as the level of correlation between the profiles of MUAPs detected over adjacent electrodes tended to  
495 1 (Figure 9A). Therefore, the high level of similarity between signals recorded from adjacent  
496 electrodes in ultra-dense grids (IED < 2 mm) may limit the percentage of identifiable motor units  
497 (Farina and Holobar, 2016). According to these results, we consider that optimal designs of surface  
498 grids of electrodes for identifying individual motor units would involve a surface that covers the  
499 muscle of interest with an IED as low as 2 mm.

500

501 Another important factor for the accuracy of the discharge times estimated for each individual motor  
502 unit is the quality of the motor unit pulse trains, estimated by the PNR (Holobar et al., 2014) or the  
503 silhouette value. In this study, we found that the quality of the identified motor units (i.e.,

504 decomposition accuracy) increased when increasing the density of electrodes or the size of the grid,  
505 with PNR reaching on average 37-38 dB across participants with the grid of 256 electrodes (Figure 4-  
506 3). A greater average PNR implies the need of less manual editing following the automatic  
507 decomposition (Hug et al., 2021b). The better estimates of motor unit pulse trains depend on the better  
508 signal to noise ratio following the inversion of the mixing matrix, since the pulse train of each motor  
509 unit is computed by projecting the extended, whitened signals on the separation vector (Holobar and  
510 Farina, 2014; Farina and Holobar, 2016; Negro et al., 2016). Likewise, the PNR substantially  
511 increased after we computationally increased the number of electrodes by spatially resampling the  
512 EMG signals. This practical result is of interest for most of the physiological studies that require a  
513 lengthy processing time to visually inspect and manually edit the discharge times estimated from the  
514 pulse trains of all the motor units (Hug et al., 2021b).

515

516 Finally, we increased both the total number and the percentage of early recruited motor units identified  
517 by independently decomposing subsets of 64 electrodes within the grids of 256 electrodes, compared  
518 to the simultaneous decomposition of all available observations (Figure 7B, C). This was likely due to  
519 the lower ratio of large motor units sampled by each subset of electrodes, allowing the algorithm to  
520 converge to smaller motor units that contributed to the signal (Figure 7B, C). Importantly, it should be  
521 noted that the simulation results were obtained independently of a specific decomposition algorithm,  
522 as previously proposed by Farina et al (2008). On the other hand, the experimental results are based on  
523 a specific algorithm. Interestingly, however, the simulation and laboratory results were fully consistent  
524 and in agreement, indicating that the difference in shape of the spatially sampled MUAPs is the main  
525 factor influencing EMG decomposition.

526

### 527 Conclusion

528 By increasing the density and the number of electrodes, and the size of the grids, we increased the  
529 number of theoretically identifiable and experimentally identified motor units from the surface EMG  
530 signals. The identified motor units had pulse trains with high PNR, limiting the manual processing  
531 time. Moreover, we identified a higher percentage of early recruited motor units, which are classically  
532 filtered out with the conventional grid designs. In this way, a maximum of 79 motor units (PNR > 28  
533 dB; mean: 36 dB), including 40% of early recruited motor units, were identified, which is substantially  
534 greater than the samples previously reported with smaller and less dense grids. From these results, we  
535 encourage researchers to develop and apply larger and denser EMG grids to cover the muscle of  
536 interest with IEDs as small as 2 mm. This approach should increase the sample of motor units that can  
537 be experimentally investigated with non-invasive techniques.

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612 **Figure Legends**

613 Figure 1: The eight grid configurations considered in this study. From the first grid of 256 electrodes  
 614 (A, grid size: 36 cm<sup>2</sup>, IED: 4 mm), six shallower and smaller grids (B-G) were artificially obtained by  
 615 discarding the relevant electrodes. (B,C,D) Density analysis: 8, 12, and 16mm IED. (E,F,G) Size  
 616 analysis: 7.7, 3.6, and 2 cm<sup>2</sup> surface area. (H) The ultra-dense prototyped grid of 256 electrodes (grid  
 617 size: 9 cm<sup>2</sup>, IED: 2 mm).

618

619 Figure 2: Results from the 200 simulated motor units with 84 configurations of grids of electrodes. (A)  
 620 Each solid line represents a motor unit territory, the scatters being the muscle fibers. Blues lines are  
 621 the theoretically identifiable motor units with a grid of 21.6 cm<sup>2</sup> and an interelectrode distance (IED)  
 622 of 18 mm, while the orange lines are the motor units revealed with a grid of 21.6 cm<sup>2</sup> and an IED of  
 623 2mm. Grey lines represent the non-identifiable motor units. The percentage of theoretically  
 624 identifiable motor units (B) and their distance from the skin (C) are reported for the 84 configurations.

625

626 Figure 3: Discharge times of the maximum number of motor units identified in one participant (S1) at  
 627 30% (A) and 50% MVC (B), with 79 and 58 identified motor units, respectively. The motor units were  
 628 identified with separated decompositions of the four grids of 64 electrodes (4 mm IED). (C) Discharge  
 629 times of the 30 first recruited motor units during the ascending ramp of force (black curve) at 30%  
 630 MVC (black box in A).

631

632 Figure 4: Effect of the electrode density on the number of identified motor units  $N$  at 30% (A, B) and  
 633 50% MVC (C, D). The boxplots in the left column report the absolute number  $N$  of identified motor  
 634 units per participant (grey dots) and the median (orange line), quartiles, and 95%-range across  
 635 participants. In the right column, the normalized number of motor units  $\bar{N}$  logarithmically decreases  
 636 with interelectrode distance  $d$  (4, 8, 12, and 16mm in abscissa) as  $\bar{N} = 195 - 68 \log(d)$  ( $r^2 =$   
 637  $1.0, p = 2.5 \cdot 10^{-5}$ ) at 30% MVC (B) and  $\bar{N} = 196 - 71 \log(d)$  ( $r^2 = 0.99, p = 0.001$ ) at 50%  
 638 MVC (D). The standard deviation of  $\bar{N}$  across subjects is displayed with vertical bars. Moreover, the  
 639 quality of the motor unit pulse trains (i.e., decomposition accuracy, estimated by the PNR) increased  
 640 when increasing the density of electrodes (see Figure 4-3 for more details). Two decomposition  
 641 procedures were considered for the 256-electrode condition; the grid of 256 black electrodes indicates  
 642 that the 256 signals were simultaneously decomposed and the grid of 256 electrodes of four different  
 643 colors indicates that four subsets of 64 electrodes were decomposed. To maintain consistency with the  
 644 computational study, the trendlines were fitted with the 4\*64 condition, which returned the higher  
 645 number of identified motor units (see Figure 4-1 for the other fitting condition). It is worth noting that  
 646 computationally increasing the density of electrodes by resampling the EMG signals with a spatial  
 647 interpolation did not reveal any previously hidden motor units (Figure 4-2).

648

649 Figure 5: Effect of the size of the grid on the number of identified motor units  $N$  at 30% (A, B) and  
 650 50% MVC (C, D). The boxplots in the left column report the absolute number  $N$  of identified motor  
 651 units per participant (grey dots) and the median (orange line), quartiles, and 95%-range across  
 652 participants. In the right column, the normalized number of motor units  $\bar{N}$  logarithmically decreases  
 653 with the size of the grid  $s$  (2, 3.8, 7.7, and 36 cm<sup>2</sup> in abscissa) as  $\bar{N} = -20 + 33 \log(s)$  ( $r^2 =$   
 654  $0.99, p = 3.0 \cdot 10^{-4}$ ) at 30% MVC (B), and  $\bar{N} = -19 + 32 \log(s)$  ( $r^2 = 0.98, p = 0.001$ ) at 50%

655 MVC (D). The standard deviation of  $\bar{N}$  across subjects is displayed with vertical bars. Moreover, the  
 656 quality of the identified motor unit pulse trains (i.e., decomposition accuracy, estimated by the PNR)  
 657 increased when increasing the size of the grid (see Figure 4-3 for more details). Two decomposition  
 658 procedures were considered for the 256-electrode condition; the grid of 256 black electrodes indicates  
 659 that the 256 signals were simultaneously decomposed and the grid of 256 electrodes of four different  
 660 colors indicates that four subsets of 64 electrodes were decomposed. To maintain consistency with the  
 661 computational study, the trendlines were fitted with the 4\*64 condition, which returned the higher  
 662 number of identified motor units (see Figure 4-1 for the other fitting condition).

663

664 Figure 6: Effect of the number  $n$  of electrodes on the normalized number  $\bar{N}$  of identified motor units at  
 665 30% (A) and 50% MVC (B). The discrete results per participant are displayed with grey data points.  
 666 The average values  $\bar{N}$  per condition are displayed with black crosses. Weighted logarithmic trendlines  
 667 were fitted to the data and returned (A)  $\bar{N} = -104 + 37 \log(n)$  ( $r^2 = 0.98, p = 0.018$ ), and (B)  
 668  $\bar{N} = -113 + 38 \log(n)$  ( $r^2 = 0.95, p = 0.016$ ). Two decomposition procedures were considered  
 669 for the 256-electrode condition; the grid of 256 black electrodes indicates that the 256 signals were  
 670 simultaneously decomposed and the grid of 256 electrodes of four different colors indicates that four  
 671 subsets of 64 electrodes were decomposed. To maintain consistency with the computational study, the  
 672 trendlines were fitted with the 4\*64 condition, which returned the higher number of identified motor  
 673 units (see Figure 4-1 for the other fitting condition).

674

675 Figure 7: (A) Typical frequency distribution of motor unit force recruitment thresholds in a human  
 676 TA. The black dashed lines denote the theoretical portions of the population of motor units recruited at  
 677 30% and 50% MVC. Effect of the grid density (B, D, F) and grid size (C, E, G) on the percentage of  
 678 early recruited motor units identified at 30% (B, C, F, G) and 50% MVC (D, E). The boxplots report  
 679 the results per participant (grey dots) and the median (orange line), quartiles, and 95%-range across  
 680 participants. (F) At 30% MVC, the percentage of early recruited identified motor units logarithmically  
 681 decreases with interelectrode distance  $d$  (4, 8, 12, and 16mm in abscissa) as  
 682  $44.6 - 13.1 \log(d)$  ( $r^2 = 0.91, p = 2.8 \cdot 10^{-3}$ ). (G) At 30% MVC, the percentage of early recruited  
 683 identified motor units does not vary with the size of the grid  $s$  (2, 3.8, 7.7, and 36  $\text{cm}^2$  in abscissa), the  
 684 logarithmic trendline fitting ( $20.5 + 1.2 \log(s)$ ) returning a negligible slope and low  $r^2 =$   
 685  $0.28$  ( $p = 8 \cdot 10^{-4}$ ). The standard deviation across subjects is displayed with vertical bars. Two  
 686 decomposition procedures were considered for the 256-electrode condition; the grid of 256 black  
 687 electrodes indicates that the 256 signals were simultaneously decomposed and the grid of 256  
 688 electrodes of four different colors indicates that four subsets of 64 electrodes were decomposed. To  
 689 maintain consistency with the computational study, the trendlines were fitted with the 4\*64 condition,  
 690 which returned the higher number of identified motor units (see Figure 4-1 for the other fitting  
 691 condition). We did not report the results when five or fewer motor units were identified in one  
 692 condition for three or more participants.

693

694 Figure 8: Effect of the electrode density on the correlation  $\rho$  between the profiles of motor unit action  
 695 potentials (MUAP) detected over adjacent electrodes (A) at 30% (B) and 50% MVC (C). The profile  
 696 of the MUAP detected over the red electrode was compared to those detected over the four adjacent  
 697 electrodes separated by a 4 (orange), 8 (blue), 12 (green) and 16 (purple) mm IED (A). The boxplots  
 698 denote the correlation coefficient  $\rho$  per participant (grey dots) and the median (orange line), quartiles,  
 699 and 95%-range across participants.

700

701 Figure 9: Results for the ultra-dense prototyped grid (2 mm IED, 5 x 1.8 cm, 256 electrodes). (A)  
 702 Description of the ultra-dense grid, where grey circles represent the electrodes. On average, the  
 703 correlation between the profiles of MUAPs detected over electrodes separated by an IED of 2 mm  
 704 (orange), 4 mm (blue), and 8 mm (purple) reached  $\rho = 0.98, 0.96,$  and  $0.92$  at 30% MVC, respectively,  
 705 and  $0.93, 0.88,$  and  $0.85$  at 50% MVC, respectively. (B) Series of discharge times for motor units  
 706 identified at 30% (left) and 50% MVC (right). The dark ticks represent the discharge times identified  
 707 with a grid of electrodes with an 8-mm IED. The discharge times in blue were additionally identified  
 708 with a grid of electrodes with a 4-mm IED, and the discharge times in red were additionally identified  
 709 with a grid of electrodes with a 2-mm IED. All the pulse trains identified with one grid were also  
 710 identified with the denser grids. (C) Effect of electrode density on the number of identified motor units  
 711 at 30% (scatters) and 50% MVC (triangles). The trendlines from the density analysis in Figure 4B, D  
 712 are also reported (red dotted lines). To maintain consistency with the other results, the grid was  
 713 decomposed as four independent subsets of 64 electrodes, as explained in the Methods, to identify the  
 714 higher number of motor units.  
 715

716 Figure 4-1. Effect of the density of the grid (A, D), the size of the grid (B, D), and the number of  
 717 electrodes (C, F) on the normalized number  $\bar{N}$  of identified motor units at 30% (A, B, C) and 50%  
 718 MVC (D, E, F).  $\bar{N}$  was estimated after decomposing the full grid of 256 electrodes and manually  
 719 editing the motor unit pulse trains. Vertical bars (A, B, D, E) are the standard deviation of  $\bar{N}$  across  
 720 subjects, scatters are the individual data points, and crosses are their mean (C, F). Logarithmic  
 721 trendlines were fitted between the averaged values  $\bar{N}$  and IED, grid size, and number of channels, as in  
 722 Figures 4, 5, and 6 of the main document. Here, the trendlines were fitted with the values obtained  
 723 from the decomposition of the full grid of 256 electrodes. Consistent with the results provided in the  
 724 main document,  $\bar{N}$  increased with electrode density ( $d$ ), grid size ( $s$ ), and with the number of  
 725 electrodes ( $n$ ) following statistically significant logarithmic trendlines ( $p < 0.05$ ). At 30% MVC,  
 726  $\bar{N} = 198 - 67 \log(d)$  ( $r^2 = 0.92$ ),  $\bar{N} = -10 + 31 \log(s)$  ( $r^2 = 0.98$ ), and  $\bar{N} = -78 +$   
 727  $32 \log(n)$  ( $r^2 = 0.90$ ). At 50% MVC,  $\bar{N} = 204 - 69 \log(d)$  ( $r^2 = 0.92$ ),  
 728  $\bar{N} = 5 + 28 \log(s)$  ( $r^2 = 0.98$ ), and  $\bar{N} = -57 + 29 \log(n)$  ( $r^2 = 0.90$ ). It is noteworthy that the  
 729 trendlines exhibited more pronounced plateaus (lower  $b$  value in the  $y = a + b \cdot \log(x)$  trendlines)  
 730 with the decomposition of the full grid of 256 electrodes than with the decomposition of subsets of 64  
 731 electrodes.  
 732

733 Figure 4-2. Correlation  $\rho$  between experimentally recorded (Left, black) and interpolated (Right,  
 734 green) EMG signals (Right, black). Using the ultra-dense grid of 256 electrodes (2-mm IED) at 30%  
 735 MVC, we spatially interpolated down-sampled montages of 4x9 electrodes with an IED of 8 mm and  
 736 5x13 electrodes with an IED of 4 mm to generate 5x13 (4-mm IED) and 10x26 (2-mm IED) grids of  
 737 electrodes, respectively. In these interpolated grids, 25% of the signals were therefore experimentally  
 738 recorded (Right, black) and 75% interpolated (Right, green). After comparing interpolated and  
 739 experimentally recorded grids of electrodes, we observed that a better signal reconstruction was  
 740 obtained with the 2-mm IED, with a correlation coefficient of  $\rho = 0.93 \pm 0.09$  between recorded and  
 741 interpolated signals. We identified 4 and 19 motor units from the interpolated grid with a 4-mm and 2-  
 742 mm IED, respectively, vs. 19 and 24 motor units with the experimentally recorded signals. We only  
 743 identified the same motor units as identified with the original less dense grids used to generate the  
 744 interpolation. These results indicate that interpolation is not sufficient to reconstruct signals from a  
 745 lower spatial sampling. This may be due to the spatial bandwidth which is greater than the inverse of  
 746 the minimal interelectrode distance used or to the edge effects of the interpolation due to the relatively  
 747 small size of the grid.

748

749 Figure 4-3. Effect of the electrode density (A, C) and grid size (B, D) on the average PNR across the  
750 identified spike trains at 30% MVC (A, B) and 50% MVC (C, D). The boxplots report the average  
751 PNRs per participant (grey dots) and the median (orange line), quartiles, and 95%-range across  
752 participants. We calculated the average PNR value for the motor unit spike trains (PNR > 28 dB)  
753 identified in each subject and condition. The average PNR across identified motor units increased  
754 together with both the density and the size of the grid. The lowest PNR values were observed with 16  
755 mm-IED ( $30 \pm 1.8$  dB at 30% MVC and  $29 \pm 1.2$  dB at 50% MVC) and with a grid of  $2 \text{ cm}^2$  ( $31 \pm 0.9$   
756 dB at 30% MVC and  $30 \pm 0.9$  dB at 50% MVC). The highest PNR was observed with 4 mm-IED and  
757 a grid of  $36 \text{ cm}^2$  ( $36 \pm 0.7$  dB at 30% MVC and  $37 \pm 0.7$  dB at 50% MVC), enabling the operators to  
758 quickly edit the identified motor units.





















