1	Performances of One-Dimensional Sonomyography and Surface			
2	Electromyography in	Tracking Guided Patterns of Wrist Extension		
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25 Abstract

26 Electromyography (EMG) and ultrasonography have been widely used for skeletal muscle assessment. Recently, it has been demonstrated that the muscle thickness change 27 collected by ultrasound during contraction, namely sonomyography (SMG), can also be 28 used for assessment of muscles and has the potential for prosthetic control. In this study, 29 the performances of one-dimensional sonomyography (1-D SMG) and surface EMG 30 31 (SEMG) signal in tracking the guided patterns of wrist extension were evaluated and 32 compared, and the potential of 1-D SMG for skeletal muscle assessment and prosthetic control was investigated. Sixteen adult normal subjects including eight males and eight 33 females participated in the experiment. The subject was instructed to perform the wrist 34 extension under the guidance of displayed sinusoidal, square and triangular waveforms at 35 36 movement rates of 20, 30, 50 cycles per minute. SMG and SEMG root mean squares (RMS) were collected from the extensor carpi radialis respectively and their RMS errors 37 38 in relation to the guiding signals were calculated and compared. It was found that the mean RMS tracking errors of SMG under different movement rates were 18.9±2.6% 39 (mean \pm SD), 18.3 \pm 4.5%, and 17.0 \pm 3.4% for sinusoidal, square, and triangular guiding 40 waveforms, while the corresponding values for SEMG were 30.3±0.4 %, 29.0±2.7%, and 41 24.7±0.7 %, respectively. Paired t-test showed that the RMS errors of SMG tracking were 42 43 significantly smaller than those of SEMG. Significant differences in RMS tracking errors of SMG among the three movement rates (p<0.01) for all the guiding waveforms were 44 also observed using one-way ANOVA. The results suggest that SMG signal, based on 45 46 further improvement, has great potential to be an alternative method to SEMG to evaluate muscle function and control prostheses. 47

Keywords- ultrasound, sonomyography, SMG, electromyography, EMG, muscle,
prosthetic control

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51 INTRODUCTION

52 Both electromyography (EMG) and ultrasonography have been widely used to detect the skeletal muscle properties and movements during static and dynamic contractions. 53 EMG describes the bioelectrical properties of skeletal muscles and reveals the 54 physiological process of the muscle contraction. It is generated by the irregular 55 56 discharges of active motor unit (MU) during the muscle activation (Zwarts and Stegeman 2003). The root mean square (RMS) magnitude of EMG is commonly used to describe 57 the time-domain information of EMG signal (Karlsson and Gerdle 2001). However, 58 despite its wide applications in different areas, EMG has some inherent limitations. It is 59 difficult for surface EMG to detect the deep muscles non-invasively, due to the fact that 60 61 the deep muscle EMG may be attenuated more and mixed by the superficial muscle EMG when reaching the skin surface. EMG signals could vary seriously from people to people 62 63 even performing the same task (Balogh et al. 1999) and be influenced by many factors, 64 such as muscle cross talk (De Luca 2002), and interelectrode distance (Alemu et al. 2003). In addition, commercially available upper-limb externally powered prosthetic devices 65 66 using EMG are still limited to one or few degrees of freedom (DoFs) (Zecca et al. 2002). 67 On the other hand, some alternative approaches have been investigated to generate signals for control purposes, including surface electroencephalography (EEG) (Heasman 68 et al. 2002), collected using embedded neurochip implants (Nicolelis 2001; Taylor et al. 69 2002); acoustic signals generated by muscles (Oster 1984; Bolton et al. 1989; Orizio et al. 70 71 1993), muscle dimensional change (Almstrom and Kadefors 1972; Kenny et al. 1999),

and tendon motions (Abboudi et al. 1999; Curcie et al. 2001), etc. These methods each have their own advantages and shortcomings and researchers in this field are still working hard to achieve signals for a better prosthetic control, such as to reduce the cognitive effort required of users, to provide direct feedback when performing movement, and to increase the number of degrees of freedom (DoFs).

Ultrasonography is another widely used method to measure muscle morphology 77 change and it has been used together with EMG to provide more comprehensive 78 information about the muscle activities and properties (Whittaker et al., 2007). 79 80 Researchers using ultrasound images have successfully detected the changes of muscle thickness (Sallinen et al. 2008), pennation angle (Mahlfeld et al. 2004), cross-sectional 81 areas (Reeves et al. 2004) and muscle fascicle length (Fukunaga et al. 2001) in both static 82 83 and dynamic conditions. Since skeletal muscle architecture is closely correlated with its 84 function (Lieber and Friden 2000), the ultrasound parameters have been employed to characterize muscle activities (Maganaris et al. 2001; Mademli and Arampatzis 2005). In 85 86 addition, it has been reported that the relationship between EMG and the muscle morphological changes extracted from ultrasound is almost linear only in lower range of 87 88 forces, but not in higher range of forces for tibialis anterior (Hodges et al. 2003), biceps brachii (Hodges et al. 2003; Shi et al. 2008), transversus abdominis (Hodges et al. 2003; 89 McMeeken et al. 2004), masseter muscle (Georgiakaki et al. 2007), etc. 90

We have recently proposed to use the real-time change of muscle thickness detected using ultrasound, namely sonomyography (SMG), for the prosthetic control (Zheng et al. 2006) and the assessment of muscle fatigue (Shi et al. 2007), isometric muscle contraction (Shi et al. 2008), and dynamic muscle contraction (Huang et al. 2007; Guo et

al. 2008). The real-time signal about the muscle thickness change during its contraction
detected using A-mode ultrasound was named as one-dimensional sonomyography (1-D
SMG). In this study, we compared the performances of 1-D SMG signal and surface
EMG signal in tracking the waveforms being displayed during the guided movement of
wrist extension in term of tracking accuracy. We hypothesized that 1-D SMG signal
could better follow the guided waveforms, thus may have potential as a non-invasive
method to detect skeletal muscle activities in vivo and to prosthetic control.

102 METHODS

103 A. Subjects

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104 Sixteen healthy adults, including eight males (mean±SD age= 26.3±3.4 years; body

weight = 70.3 ± 11.9 kg; height = 172.9 ± 8.5 cm) and eight females (mean \pm SD age =23.5

 ± 1.2 years; body weight = 50.4 ± 4.1 kg; height = 160.3 ± 1.7 cm), volunteered to

participate in this study and were tested within a period of two months. All the participants were right-hand-dominant without any known neuromuscular disorders. The human subject ethical approval was obtained from the relevant committee in the authors' institution and informed consents were obtained from all subjects prior to the experiment.

111 B. Data acquisition and processing

An ultrasound pulser/receiver (model 5052 UA, GE Panametrics, Inc. West Chester, OH, USA) was used to drive a 10 MHz single element ultrasound transducer (model V129, GE Panametrics, Inc., West Chester, OH, USA), and to amplify the received signals. The A-mode ultrasound signal was digitized by a high speed A/D converter card

with a sampling rate of 200 MHz (Gage CS82G, Gage Applied Technologies, Inc, 116 117 Canada). The surface EMG signal, captured from the EMG bipolar Ag-Agcl electrodes (Axon System, Inc., NY, USA), was amplified by a custom-designed EMG amplifier 118 119 with a gain of 1000 and filtered by a 10-300 Hz band-pass analog filter within the 120 amplifier, and then digitized by a data acquisition card (NI-DAQ 6024E, National 121 Instruments Corporation, Austin, TX, USA) with a sampling rate of 4 KHz. The A-mode 122 ultrasound signal was saved frame by frame together with surface EMG for subsequent 123 analysis in a PC with 2.8 GHz Pentium IV microprocessor and 512 MB RAM. The frame 124 rate of A-mode ultrasound was approximately 17 Hz, which was also applied to the data 125 rates of SMG and EMG RMS signals.

The 10 MHz single element ultrasound transducer (radius 3 mm) was inserted into a 126 127 custom-designed holder (radius 10 mm) made of silicone gel in order to attach the 128 transducer to the skin stably (Fig. 4). The transducer together with the holder was positioned on the skin where the belly of extensor carpi radialis is. Double-sided adhesive 129 130 tape was used to fixate the holder, while ultrasound gel was imposed between the transducer and skin. The EMG bipolar Ag-Agcl electrodes were attached to the skin 131 132 surface near the ultrasound transducer and along the extensor carpi radialis muscle. The distance between the two electrodes was approximately 20 mm and an additional 133 electrode for providing the reference electrical signal was placed near the head of ulna. 134

The A-mode ultrasound and surface EMG were collected, stored and analyzed by the software for ultrasound measurement of motion and elasticity (UMME, http://www.sonomyography.org) developed using Visual C++. The time delay between the two data collection systems was calibrated using a method similar to that described by

Huang et al. (2005, 2007). As the transducer moved cyclically up and down in a water
tank, the two signals representing A-mode ultrasound, and simulated EMG respectively
were collected and stored. The time delay between the data sets was calculated using a
cross-correlation algorithm. The details can be found in our earlier study (Huang et al.
2007).

The muscle deformation signal, i.e. SMG, was extracted from the A-mode ultrasound. A cross-correlation algorithm was employed to track the displacements of upper and lower boundaries of extensor carpi radilis muscle during the wrist extension. The equation used to calculate the normalized one-dimensional cross-correlation is as follow:

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$$R_{xy} = \frac{\sum_{i=0}^{N-1} [x(i) - \bar{X}][y(i) - \bar{Y}]}{\sqrt{\sum_{i=0}^{N-1} [x(i) - \bar{X}]^2 \sum_{j=0}^{N-1} [y[j] - \bar{Y}]^2}}$$
(1)

Where \overline{X} and \overline{Y} are the means of x(i) and y(j), respectively. It requires a reference 149 signal from an initial frame and would search for the signal most similar to the reference 150 signal for estimating the object position in the updated frame. The A-mode ultrasound 151 echoes reflected from the fat-muscle and muscle-bone interfaces were selected by two 152 153 tracking windows (Fig. 2c) in the first frame. When the muscle was contracting, its dimensional changes induced the variations of distance between the interface of fat-154 muscle and that of muscle-bone, which would cause the A-mode ultrasound echoes to 155 156 shift for a certain distance. The percentage deformation of the muscle is defined as

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$$D = \frac{(d - d_0)}{d_0} \times 100\%$$
(2)

158 Where d_0 is the initial distance between the two echoes and d is the distance when the

159 muscle is contracting.

160 The RMS amplitude of EMG was calculated and compared with the SMG signal to 161 investigate which one could better follow the guiding waveforms during the wrist 162 extension.

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164 *C. Experiment protocol*

165 Before the experiment formally began, all the subjects were trained for two or three trials to make sure they were familiar with the experimental protocol. None of subject had 166 been trained before. Both 1-D SMG and surface EMG signals were tested for their 167 168 accuracy in following the displayed waveform patterns. As shown in Fig. 1, the subject was seated comfortably in an adjustable chair with his/her trunk fixed by a strap onto the 169 back of the chair to prevent posture change during the test and the right forearm resting 170 171 on the table with pronation. The elbow was flexed at approximately 140 degree between the upper arm and forearm. The angle between the upper arm and trunk was 172 approximately 30 degrees. The subject was instructed to perform wrist extension under 173 the guidance of displayed sinusoidal (Fig. 2a), square (Fig. 2d) and triangular waveforms 174 175 (Fig. 2e) respectively. The order of the experiments was randomly selected for each subject. For the SMG test, the subject was required to perform several wrist extensions 176 before each experiment in order to determine the amplitude of the muscle deformation 177 signal extracted from A-mode ultrasound (i.e. 1-D SMG), and the amplitudes of the 178 guiding waveforms were adjusted based on the obtained muscle deformation range. 179 During the experiments, the subjects were encouraged to try their best to produce real-180 181 time muscle deformation signal, i.e. SMG, the same as the waveform being displayed on

the screen by adjusting the range of their wrist movement in response to the visual 182 183 feedback from the guiding waveforms. If the muscle deformation signal generated did not follow the guiding waveform well, the subjects could adjust the strength of their muscles 184 in order to match the two waveforms better. The wrist extension rates were set to be 20, 185 30, 50 cycles per minute for each guiding waveform. Therefore, every subject totally 186 187 performed nine tasks of wrist extension for the three different movement patterns 188 (sinusoidal, square and triangular waveforms) for SMG tests (Fig. 2). Three repeated 189 trials were performed for each task and there was a rest of 3 minutes between two adjacent trials to avoid muscle fatigue. The A-mode ultrasound signals were saved in the 190 191 PC hard disk for further analysis. To make the system response time comparable to the subsequent EMG test, the EMG signals were also collected and analyzed during the 192 ultrasound measurement but the EMG RMS signal was not displayed and the results not 193 used. 194

The subjects were also instructed to perform another set of wrist extension tasks, using 195 196 the RMS of their surface EMG signals to follow the reference waveforms. Similar testing 197 protocol was adopted as that in the SMG test. To make the results comparable, during the EMG test, the A-mode ultrasound signals were collected and analyzed in real-time but 198 199 the SMG signal was not displayed, as shown in Fig. 3. The subjects could adjust the 200 range of wrist movement according to the real-time display of their EMG RMS signals to better fit the reference signal. Totally nine tasks of wrist extension for surface EMG test 201 202 under the three wrist extension rates for the three different waveforms were performed by 203 each subject. Figure 3 shows the interface of the software to collect the data of EMG RMS and the three types of guiding waveforms. 204

205 D. Data analysis

The SMG and EMG RMS data were respectively normalised by expressing measures as a percentage of the largest SMG and EMG RMS signals detected any time during the testing procedure. The RMS tracking errors (RMSTE) between SMG/EMG RMS and the corresponding guiding waveforms were calculated separately, defined as:

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$$\text{RMSTE} = \sqrt{\frac{1}{N} \sum_{n=1}^{N} (Sig_1(n) - Sig_2(n))^2}$$
(3)

211 where $Sig_1(n)$, $Sig_2(n)$ are signals with N points of values.

The performances of SMG and EMG RMS to follow the three guiding waveform patterns were compared using paired t-test. One-Way ANOVA was also used to determine whether there were any differences in the performances of the SMG signals under the three different movement rates. All the data were calculated using Minitab (Minitab Inc., Pennsylvania, USA). Statistical significance was set at the 5% probability level.

218 **RESULTS**

Totally 432 data sets were recorded from the sixteen subjects. Table 1 summarizes the RMS tracking errors of SMG and EMG for the three guiding waveform patterns under different movement rates. The overall mean RMS tracking errors of SMG under the three movement rates were $18.9\pm2.6\%$ (mean \pm S.D.), $18.3\pm4.5\%$, and $17.0\pm3.4\%$ for the sinusoid, square, and triangle guiding waveforms, while the corresponding values for EMG were $30.3\pm0.4\%$, $29.0\pm2.7\%$, and $24.7\pm0.7\%$, respectively (Fig. 5). Paired t-test revealed that the overall mean RMS tracking error of SMG was significantly smaller than
that of EMG for all the three guiding waveforms.

One-way ANOVA showed that there were significant differences in the RMS 227 tracking errors of SMG among the three wrist extension rates (p<0.01) for all the three 228 guiding waveforms as demonstrated in Fig. 6. An apparent increasing trend of the RMS 229 230 tracking error using SMG was observed with the increase of the movement rate for all the 231 different guiding patterns. However, for EMG, statistical analysis revealed that the RMS tracking error was significantly different among the three movement rates only for the 232 233 square waveform (P=0.001), but not for sinusoid (P=0.921) and triangle (P=0.762) waveforms. As shown in Fig. 7, the RMS tracking error for EMG generally showed 234 smaller variations under different rates of wrist extension. 235

236 **DISCUSSION**

237 In this paper, we investigated the performances of surface EMG and 1D SMG, i.e. real-time muscle thickness change detected using A-mode ultrasound, in tracking three 238 different movement patterns of the wrist extension guided by waveforms shown on the 239 PC screen. We found that the tracking errors of SMG under different wrist extension 240 rates (ranged from $14.0\pm1.9\%$ to $23.3\pm3.7\%$) were statistically significantly smaller than 241 242 the corresponding values of surface EMG (ranged from $24.2\pm4.4\%$ to $32.1\pm4.1\%$) for all 243 the movement patterns studied (Fig. 5 and Table 1), indicating that SMG performed 244 better than surface EMG in following the given movement patterns in term of tracking 245 accuracy.

For decades, EMG signal has been widely used in the areas of muscle fatigue 246 247 (Masuda et al. 1998; Fukuda et al. 2006), muscle pathology (Haig et al. 1996; Hogrel 2005; Labarre et al. 2006; Ohata et al. 2006), prosthetic device control (Kermani et al. 248 249 1995; Boostani and Moradi 2003; Soares et al. 2003), and athlete muscle assessment under different postures (Worrell et al. 1992). Some researchers have demonstrated that 250 251 the relationship between EMG and the force of the related joint was not linear (Alkner et 252 al. 2000). Whereas, in our previous study, it was shown that the SMG signal was linear 253 with the torque generated by biceps brachii muscles (Shi et al. 2008). These results may 254 indicate that SMG signal may have a more direct, simple correlation (linear) with the 255 torque generated by the corresponding muscle. The results of this study further demonstrate the potentials for SMG to serve as a feedback of rehabilitation of muscle 256 257 dysfunction and assessment of muscle activity...

258 Compared with surface EMG, the main advantage of SMG is that ultrasound can inherently detect individual muscle at neighbouring locations and different depths 259 without the effects of muscle cross talk by using one or more ultrasound transducers. Due 260 to the challenges in separating SEMG signals generated by different neighbouring 261 262 muscles, i.e. cross talk, the available prostheses controlled by SEMG could only provide limited number of DoFs. By using multi-channels of SMG signal, it is possible to realize 263 the control of prostheses with multiple DoFs. It may benefit the users with more grasping 264 functions and less training efforts. 265

Further studies are required to demonstrate these advantages quantitatively. It is also very interesting to further investigate whether the good performance of SMG on the extensor carpi radialis muscle for wrist control observed in this study can be applied to

other skeletal muscles. A follow-up study using SMG to control real powered prostheses
with a hand open-close feature is being conducted in our group. It has already been
demonstrated that the muscles of residual limbs could generated SMG as well (Zheng et
al. 2006).

It is interesting to explore why SMG could perform significantly better than EMG in 273 274 tracking different given movement patterns of the joint under different movement rates. It 275 has been reported that there is an exponential relationship between EMG magnitudes and the strengths generated by different skeletal muscles (Deluca 1997; Hodges et al. 2003; 276 277 Zheng et al. 2006; Shi et al. 2008). We have previously found that SMG signals of a skeletal muscle have approximately linear relationships with the strengths generated by 278 this muscle, represented either by torques for isometric contractions (Shi et al. 2008) or 279 280 by joint angles for isotonic contractions (Zheng et al. 2006). It appears that SMG and the 281 corresponding joint angle follow a relatively simple relationship in comparison with the 282 relation between EMG and joint angle. The results of this and previous studies appear to 283 imply that the architectural changes during muscle contraction relate more directly to the actuation achieved (mechanical output), while the EMG is a measure of activation 284 285 intended (electrical input). In relation to the findings of the present study, we may interpret that our motor control and visual feedback system could perform better when the 286 control signal has a linear relationship with the target signal to control, which is the wrist 287 angle in this case. This may probably reduce the training efforts when the SMG signal is 288 used for the prosthetic control. Further studies are required to study how many training 289 290 efforts can be saved when using SMG for control instead of EMG. More normal and residual limbs should be tested to ensure a solid conclusion. 291

As expected, it was found that as the movement rates increased, the tracking errors of 292 293 SMG increased (Fig. 6). When the movement rates increased, the subjects were required to perform the same movement in a shorter time period. Furthermore, according to 294 295 subjects' verbal reports, the visual feedback used to provide instantaneous performance 296 indication during the test slightly distracted their attention. Thus, as the movement rates increased, the possibility for SMG to "move away" from the guiding waveform may also 297 298 increase, resulting in higher tracking errors. However, this increasing trend in tracking 299 error with the increase of movement rate was not observed in EMG RMS (Fig. 7). It was also noted that the performance of SMG tracking at the highest rate was still better than 300 301 the best performance of the EMG tracking among all the tests. The reducing performance of SMG induced by the increase of movement speed may have a number of potential 302 reasons. First, the frame rate of A-mode ultrasound (approximately 17 Hz), which also 303 determines how fast the data points of SMG signal are given, was relatively low in the 304 305 study. With the increase of the wrist flexion-extension rate, the SMG data collected in each cycle would be reduced. Therefore, the subject may have fewer data points to refer 306 to for following the given waveform. Since we have also controlled the data rate of EMG 307 308 RMS to 17 Hz during the test, this effect should have affected the performance of EMG tracking as well when the movement speed was increased, however, it was not observed 309 in this study. A higher frame rate system could be used to further investigate the effect of 310 data collection speed in future studies. The second possible reason is that SMG is a signal 311 not only related to the bioelectrical properties of muscles, i.e. how muscles are activated, 312 but also dependent on the mechanical properties of muscle-tendon complex, i.e. 313 314 viscoelastic properties. With the increase of the muscle contraction speed, the hysteresis

of SMG signal may also increase. This will make it more challenging for the subjects to follow the given movement patterns using SMG signal. However, EMG signals would not be affected by this effect, as they are more related to bioelectrical properties of muscles. Again, further studies are required to better understand the effects of the viscoelasticity of muscles and other tissues on the generation and applications of SMG signal.

321 In summary, we demonstrated in this study that SMG signal obtained using A-mode ultrasound could provide better performance flexion-extension of wrist in comparison 322 323 with EMG in tracking different given patterns under different wrist flexion-extension rates. The use of single element transducer in A-mode image allowed great flexibility in 324 designing SMG sensor, thus it is practically feasible to attach such a probe on the skin 325 326 surface conveniently for the purposes of control or muscle function evaluation, similar to 327 the use of surface EMG. However, further studies are required to verify the performances of SMG signals on different muscles under different conditions. The 328 329 mechanism of how the increasing movement rate of wrist affects the SMG tracking performances should also be further investigated. 330

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448 **Captions of Figures**

449 Fig. 1 The diagram of the data collection system.

Fig. 2 The software interface was used to simultaneously collect 1-D SMG, SEMG 450 451 signals (a) The sinusoidal waveform with a rate of 20 cycles per minute was used to 452 guide the wrist extension movement. The subject used 1-D SMG signal to track the sinusoidal pattern. (b) Surface EMG was also collected for reference. (c) The muscle 453 454 deformation signal (i.e. SMG) was measured by detecting the distance change between 455 the A-mode ultrasound echoes reflected from the fat-muscle and muscle-bone interfaces, which were selected by two the tracking windows. A cross-correlation algorithm was 456 employed to track the movements of the echoes during the wrist extension. The muscle 457 458 deformation signal (i.e. SMG) was calculated using the change of the time interval 459 between the echoes and displayed along with the guiding waveform for tracking (a). (d) 1-D SMG signal tracks the square waveform with a rate of 30 cycles per minute; (e) 1-D 460 SMG signal tracks the triangular waveform with a rate of 50 cycles per minute. 461

Fig. 3 (a) The software interface was used to collect the SEMG and A-mode ultrasound signals. (a) SEMG signal was collected. (b) The sinusoidal waveform with a rate of 20 cycles per minute was used to guide the wrist extension movement. SEMG RMS was calculated to track the sinusoidal pattern. (c) A-mode ultrasound signal was also collected for reference (d) SEMG RMS tracks the square waveform with a rate of 30 cycles per minute; (e) SEMG RMS tracks the triangular waveform with a rate of 50 cycles per minute.

469	Fig. 4 Placen	nent of the	1D ultrase	ound	transducer,	SEMG elec	etrode	s on t	the	forea	rm, with
470	ultrasound g	gel applied	between	the	ultrasound	transducer	and	skin	to	aid	acoustic
471	coupling.										

Fig. 5 The RMS tracking errors (%) between SMG/SEMG and the guiding waveforms.
The error bar represents the standard deviation of the results of three different movement
rates.

Fig. 6 The tracking errors of SMG for the three guiding waveforms under different movement rates. The error bar represents the standard deviation of the results of the sixteen subjects.

Fig. 7 The RMS tracking errors of SEMG under the three different wrist extension rates
for different guiding waveforms. The error bar represents the standard deviation of the
results of the sixteen different subjects.

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483	Table 1. The RMS tracking errors (%) between SMG/surface EMG and the guiding
484	waveforms at the three movement rates (Mean \pm S.D.) and the mean RMS tracking errors
485	averaged over the three movement rates for sinusoid, square, and triangle guiding
486	waveforms.

Rate		SMG		Surface EMG			
(Cycles							
minute)	sinusoid	square	triangle	sinusoid	square	triangle	
20	16.3±7.8	14.6±1.7	14.0±1.9	30.5±4.7	27.0±4.2	24.2±4.4	
30	19.0±3.0	17.1±1.6	16.3±2.5	29.9±6.4	27.8±3.0	24.4±6.3	
50	21.5±3.2	23.3±3.7	20.7±3.1	30.6±5.5	32.1±4.1	25.4±4.8	
Mean	18.9±2.6	18.3±4.5	17.0±3.4	30.3±0.4	29.0±2.7	24.7±0.7	













Fig. 4









