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Tutorial. Frequency analysis of the surface EMG signal: Best practices

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ABSTRACT

This tutorial is aimed primarily to non-engineers (clinical researchers, clinicians, neurophysiology technicians, ergonomists, movement and sport scientists, physical therapists) or beginners using, or planning to use, surface electromyography (sEMG) as a monitoring and assessment tool for muscle and neuromuscular evaluations in the prevention and rehabilitation fields.

Its first purpose is to explain, with minimal mathematics, basic concepts related to: (a) time and frequency domain description of a signal, (b) Fourier transform, (c) amplitude, phase, and power spectrum of a signal, (d) sampling of a signal, (e) filtering of sEMG signals, (f) cross-spectrum and coherence between two signals, (g) signal stationarity and criteria for epoch selection, (h) myoelectric manifestations of muscle fatigue and (i) fatigue indices. These concepts are consolidated knowledge and are addressed and discussed with examples taken from the literature.

1. Introduction

This tutorial is part of a series published by the Journal of Electromyography and Kinesiology that aims to explain engineering concepts relevant for the acquisition, processing and interpretation of surface electromyographic (sEMG) signals to a readership of non-engineers (Merletti and Muceli, 2019; Del Vecchio et al., 2020; Merletti and Cerone, 2020; Clancy et al., 2023; Valli et al., 2024). This is achieved by minimizing the use of mathematical concepts and expressions. This tutorial is devoted to the frequency analysis of the sEMG signals. Specifically, we introduce the concepts of amplitude, phase, and power spectra, bandwidth, sampling frequency, cross-spectrum and coherence, and describe some consolidated applications. The frequency analysis of sEMG is important for understanding a) the process of signal conditioning and sampling (Merletti and Cerone, 2020) and b) clinical applications which are today mostly focused on the myoelectric manifestations of muscle fatigue (MMMF) in ergonomics, sport and rehabilitation sciences and on the quantification of coherence between signals.

As in the case of music, voice, or other bioelectric signals, the sEMG description in the “time and frequency domains” is analogous to the use of two different languages (domains) each of which being more suitable than the other to outline specific signal changes potentially associated to physiological or pathological conditions and able to monitor their evolution or treatment effectiveness. The time domain shows how the signal changes over time. This can be compared to using one language where the words convey meaning and information in a sequential manner. In the frequency domain, a signal is represented by showing the different frequencies (sinusoidal harmonics) present in the signal and their respective amplitudes. This can be compared to using another language where different tones convey meaning and information in a non-sequential manner. Both domains/languages offer unique insights into the nature of the signal/communication, and being proficient in both enhances our overall capability of interpretation of the signal.

The two representations are inter-related, and it is possible to switch from one domain to the other (e.g., from the time to the frequency domain, and vice versa). In the following we rehearse some fundamental concepts about the time domain signals (section 2), the concepts of frequency and Fourier transform (sections 3-4), the concepts of spectrum, cross-spectrum and coherence (sections 4 and 5), the notions of bandwidth and filtering of a signal (section 5), the concept of signal stationarity (section 6), some applications related to the MMMF (section7), and best practices related to the EMG frequency analysis (section 8). This material is presented at an intermediate level: more elementary presentations can be found in https://www.robertomerletti.it/en/emg/material/tech/ and in https://www.robertomerletti.it/en/emg/material/teaching/module4. More advanced material can be found in https://scholarworks.iu.edu/dspace/handle/2022/21365 and in (Samani, 2019).

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Nomenclature

ARV	Average rectified value
DFT	Discrete Fourier Transform
ECG	Electrocardiogram / Electrocardiography
EEG	Electroencephalogram / Electroencephalography
EMG	Electromyogram /Electromyography
FFT	Fast Fourier transform
MDF	Median frequency of the PSD
MFCV	Muscle fiber conduction velocity
MMMF	Myoelectric manifestations of muscle fatigue
MNF	Mean frequency of the PSD
MSC	Magnitude-squared coherence
MU	Motor unit
MUAP	Motor unit action potential
MVC	Maximal voluntary contraction
PLI	Power line interference
PSD	Power spectral density (power spectrum)
RMS	Root mean square value
sEMG	Surface electromyogram or Surface electromyography

2. Continuous-time vs discrete-time signals

The acronym EMG is used in this work to indicate either electromyography or electromyographic signal. Electromyography is a method to measure the electrical activity produced by muscle activation. Muscles are controlled by motoneurons at the spinal cord (or brain stem). Each motoneuron innervates a bundle of fibers (muscle unit). A motoneuron and the corresponding muscle unit form a motor unit (MU) (Liddell and Sherrington, 1925). Each motoneuron transmits, along its axon, a sequence of brief electrical signals, known as train of action potentials, to the innervated muscle fibres. Therefore, a motoneuron action potential always leads to a MU action potential (MUAP). Since, a number of MUs are recruited when a muscle is active, an EMG signal is a sum of trains of MUAPs.

An EMG signal can be measured by means of electrodes placed on the skin overlying the target muscle (surface EMG or sEMG) or by means of wire or needle electrodes inserted into the target muscle (intramuscular EMG). Surface EMG is a voltage distribution over the skin, that is an image. This image can be sampled in space by one or more electrodes (sEMG channels). A single electrode defines a pixel with surface equal to the electrode contact area and provides the time course of the average voltage under such area with respect to a reference. In fact, a single channel sEMG is the difference in electrical potential between two electrodes. When sEMG is acquired in monopolar configuration, one electrode is placed over the muscle while the other electrode (reference) is placed in a body region where muscle activity is presumably absent (e.g., on the skin overlying a bone prominence) (Fig. 1 a). When sEMG is acquired in bipolar configuration, also indicated as single differential montage, two electrodes are placed over the muscle and the difference between the two monopolar signals is computed. A third electrode is a reference for both (Fig. 1a). The monopolar and the single differential voltages change over time along with the muscle activation. The corresponding sEMG signals is defined as "continuous-time" signal (Fig. 1b, black trace) as observed on an oscilloscope (see https://www.robertomerletti.it/en/emg/material/teaching/module10). That means that the signal exists for any time value (in the time interval the signal is acquired). We indicate the signal with $v(t)$, where t is the time and can assume any real value. Other examples of analog signals are the sound picked up by a microphone, an electrocardiogram (ECG), and an electroencephalogram (EEG) or a blood pressure waveform.

In order to be able to store and process the signal in a computer, we have to transform it into a sequence of samples whose amplitude changes in time. This operation is known as "sampling". Sampling produces a discrete-time signal by taking the values of a continuous-time signal at evenly spaced points in time (Fig. 1b, blue stems). The distance between two samples is named "sampling period" (T_{samp}) (Fig. 1b, green time interval). The inverse of the sampling period is the "sampling frequency" $f_{samp} = 1/T_{samp}$, which indicates the number of samples taken per second expressed in samples/s or s^{-1} or Hz. The sampled version of the $v(t)$ is indicated with $v(t)|_{t=nT_{samp}}$ or $v[n]$, where $n = 0, 1, \cdots, N-1$, and

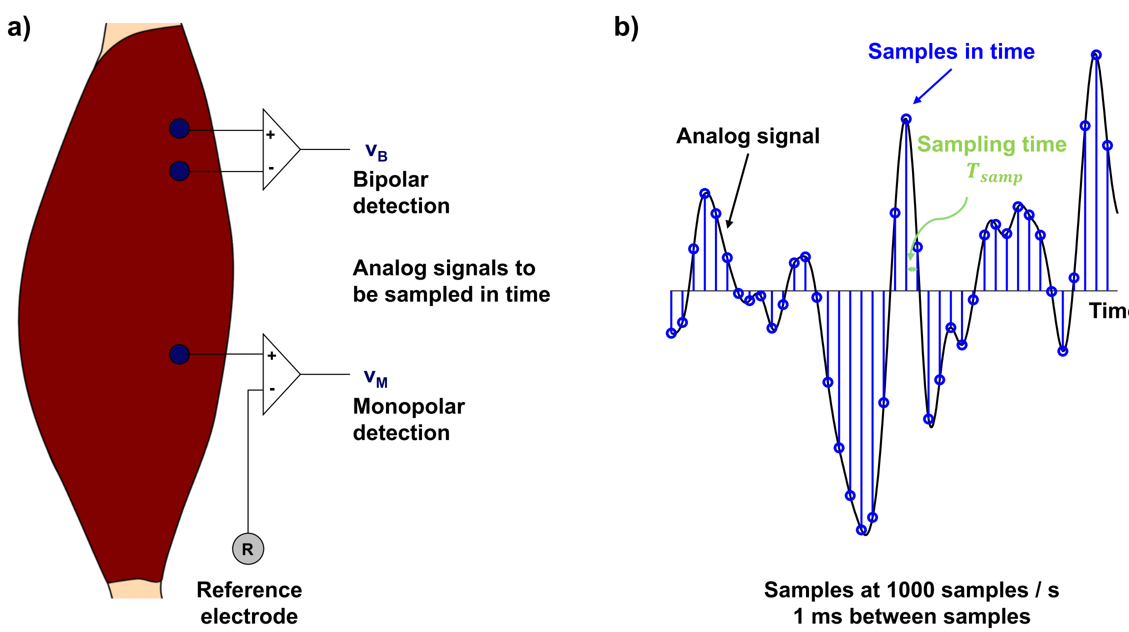

Fig. 1. a) Monopolar and bipolar detection of a surface EMG signal. In case of monopolar detection, a recording electrode is placed over the muscle of interest, and a reference is located at an electrically neutral site, while in case of bipolar (single differential) detection, both recording electrodes are placed over the target muscle. Some modern single differential detection systems do not require a reference electrode and create an internal reference. b) Example of an analog EMG signal (black trace) and the discrete signal resulting from sampling (blue stem plot). In this example, the sampling period is $T_{samp} = 1$ ms. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

N is the total number of acquired samples.

Most signals we will consider in the following are supposed to be discrete in time. Discrete signals can be represented as stem plots (as in Fig. 1b), line plots (only the vertical lines in Fig. 1b), or dotted plots (only the dots in Fig. 1b), where each dot represents the sample amplitude. Note that often a discrete-time signal is represented in graphs as a continuous line. This is due to the fact that the samples are connected by lines in the plot or the number of samples in the displayed time interval is so high that the samples are close to each other to a point that we cannot graphically distinguish individual samples (see examples in Fig. 2b and c). Sampling has to be sufficiently fast not to lose any of the information carried out by the continuous-time signal. This concept is explained in section 5.2.

3. The concept of signal frequency

Musical notes probably offer the most familiar concept of the frequency content in signals. An audio signal, including music, indicates how the air pressure in our ears changes compared to the ambient pressure value. A pure tone corresponds to a periodic sinusoidal signal $x(t)$ (Fig. 2a) that can be expressed as

$$x(t) = B + A\cos(\omega t + \varphi) = B + A\cos(2\pi ft + \varphi) = B + A\cos(2\pi t/T + \varphi) \tag{1}$$

where A, B, ω, φ , f and T are real and constant, and t is the continuous-time variable. B is the value of the ambient pressure value, A is the amplitude of the sinusoid, $\omega = 2\pi f$ is its angular frequency (radians/s), $f = 1/T$ is its frequency (Hz), φ is the phase (radians), $T = 1/f$ is the period and t is time (s). A general signal (including a sinusoid) is said to be periodic if it repeats itself after a time interval T, known as period. If T is the smallest time interval after which the signal repeats itself, it is qualified as fundamental period. For instance, if $\omega = 2\pi$ radian/s, then $f = \omega/2\pi = 1$ Hz and $T = 1/f = 1$ s. The amplitude A denotes how "loud" is the sound we perceive. Given a single tone at a certain frequency, the bigger the value of A, the louder the sound we perceive. The phase is the shift of the sinusoid with respect to the time axis.

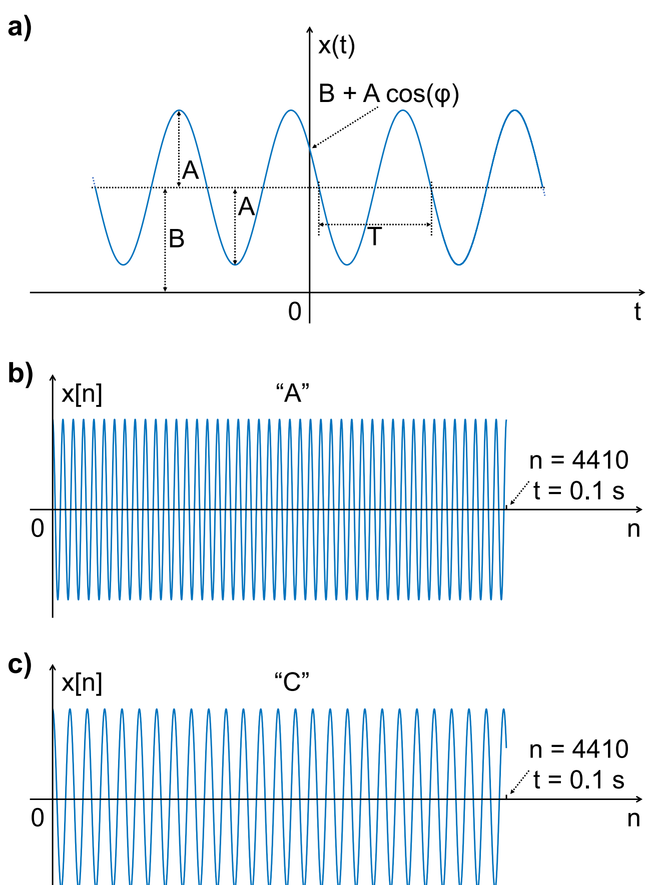

Fig. 2. A) A pure tone represented by a sinusoid with period T, amplitude A, phase φ, and offset B. b) A discrete sinusoidal signal corresponding to the pure tone "A" (440 Hz). c) A discrete sinusoidal signal corresponding to the pure tone "C" (261.6 Hz). Note that the signal in panel b oscillates faster than the signal in panel c. The sampling frequancy was 44100 samples/s.

All signals stored in a computer, including audio signals, are discrete in time. The corresponding notation is

$$x[n] = B + A\cos(\Omega n + \varphi) \tag{2}$$

where A, B, Ω, and φ are real and constant, $\Omega = \omega T_{samp} = 2\pi f/f_{samp}$, and n is an integer counting the samples. This notation may appear complicated, but $x[n]$ is simply $x(t)$ taken (sampled) at the time instants nT_{samp}, where T_{samp} is the sampling period, i.e., the difference in time between two consecutive samples, and $f_{samp} = 1/T_{samp}$. Note that a discrete sinusoidal signal $x[n]$ is periodic with period N, i.e., it repeats itself identically after N samples

$$x[n] = x[n+N] = B + A\cos(\Omega(n+N) + \varphi) \tag{3}$$

only if ΩN is a multiple integer of 2π, i.e., $\Omega N = 2\pi k$, with k and N integers.

Also, note that we have considered here the discrete-signal corresponding to the change in the air pressure. Audio signals are usually sampled at a sampling frequency of 44,100 Hz. The concept of sampling frequency is mentioned in section 2 and will be taken again in section 5.2.

Let's now assume that the ambient pressure is $B = 0$, and the phase $\varphi = 0$. Let's also consider two values of f equal to 440 Hz (Fig. 2b) and 261.6 Hz (Fig. 2c) corresponding to the A and C notes of the 4th octave.

We can notice that the sinusoid in Fig. 2b oscillates faster than the sinusoid in Fig. 2c. These examples help to visualize the concept of frequency. Rapid oscillations correspond to high frequencies, slow oscillations correspond to lower frequencies. If we listen to the corresponding sounds, we can see that "A" has higher pitch compared to "C". Although the concepts of frequency and pitch are not the same, in that the pitch is our ears' response to the frequency of a sound, there is somehow a parallel, in that high frequency tones produce high pitch sounds and low frequency tones produce low pitch sounds. For simplicity, we have considered pure tones in the former examples. However, usually, a sound is made by the combination of multiple tones, weighted with different amplitudes and shifted by different phases.

Fig. 3a shows a periodic signal $s(t)$ with period $T = 0.1$ s, that can be obtained as the sum of 4 sinusoidal components with amplitude A_1, A_2, A_3, and A_4, frequency $1/T$, $2/T$, $3/T$, and $4/T$, and phase φ_1, φ_2, φ_3, and φ_4. These 12 parameters (3 per sinusoid) define the signal in the frequency domain. We can represent the signal with this compact notation in the frequency domain with two diagrams, one for the amplitude (Fig. 3 b) and one for the phase.

The signals we mentioned so far are mono-dimensional and vary in the time domain. However, in general, signals can be multi-dimensional, and are not necessarily a function of time. For instance, high-density surface EMG electrodes provide an image of the electric potential distribution over the surface of the skin underneath the electrodes. That is a bidimensional signal, i.e., a function of two spatial coordinates, in the horizontal and vertical directions, along x and y, respectively. The concept of frequency can expand to the space domain, but this topic goes beyond the scope of this tutorial. The interested reader is referred to (Afsharipour, Soedirdjo and Merletti, 2019; Merletti and Muceli, 2019). In the next 4 sections, we will consider only "mono-dimensional" EMG signals, as obtained by a monopolar electrode or a pair of bipolar electrodes.

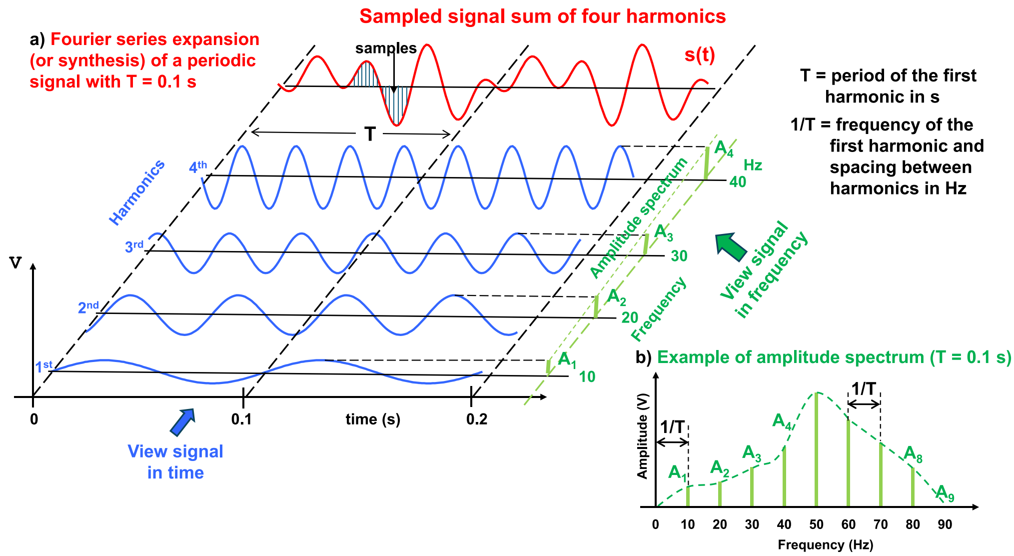

Fig. 3. a) A periodic signal $s(t)$ with period $T = 0.1$ s, obtained as sum of 4 sinusoidal components, spaced by $1/T = 10$ Hz. b) Example of an amplitude spectrum for a signal of 0.1 s duration with harmonics in the range 10 to 80 Hz. Modified from https://www.robertomerletti.it/assets/pdfs/technical_notes/technical_note_2_analysis_of_a_signal.pdf.

4. Fourier analysis

4.1. The concept of transform

We have seen that the signal $s(t)$ in Fig. 3a can be obtained as the sum of four sinusoidal components. Can we always transform a signal into a weighted sum of sinusoids? How can we visualize the transformation? When is it useful to apply it?

A transform causes a change of appearance, so that some information becomes easier to visualize, extract, and/or manipulate. A transform commonly applied to time domain signals is the Fourier transform, that converts a function (signal) into a form that describes the frequencies present in the original signal. In other words, we can state that the Fourier transform converts a signal from the time to the frequency domain. The process of decomposing a generic signal into the sum of sinusoidal components is known as Fourier analysis (or frequency analysis or spectral analysis). Examples are provided in the Technical Note #2 in https://www.robertomerletti.it/en/emg/material/tech/. The inverse process, that consists in re-building the original function from its sinusoidal components is referred to as synthesis. The process of synthesis requires the application of the inverse Fourier transform. To provide a more concrete example, let's imagine playing the two tones "A" and "C" simultaneously and recording the resulting sound signal. To determine which components are present in the signal requires computing the Fourier transform (analysis). It is possible to recreate (synthetize) the original sound by including the frequency components identified by the Fourier analysis (music synthesizer).

Now that we have learned the concept of transform, an obvious question is when its application is useful. Depending on the operation we want to apply on the signal of interest, or the information we want to extract from it, one domain may be more suitable than the other. For instance, amplification is easier in the time domain, removal of noise or interferences is easier in the frequency domain. Therefore, if the latter is our aim, we can simplify our task by calculating the Fourier transform of the signal at hand, manipulate it for our purpose and then take the Inverse Fourier transform to obtain a processed signal. We will be back to the concept of noise in section 5.3.

4.2. Fourier series

In general, the term "Fourier transform" refers to the transform of a nonperiodic continuous-time signal but different transforms with specific names exist depending on whether the signal in exam is periodic or not, continuous or discrete in time. Each of these transforms used for the analysis has a corresponding inverse transform that can be used for the synthesis after manipulation. We will now go through the most important transforms for our application, starting from the Fourier series.

In general, we can state that we can transform a signal from the time to the frequency domain if the signal is periodic. Let's first consider continuous-time signals. A continuous-time periodic signal repeats itself identically after a time interval T, defined as period.

Any periodic signal can be described as, or "expanded" into, the sum of a (potentially infinite) number of sinusoids of proper amplitude, frequency, and phase whose sum produces the original signal. We refer to this expansion of a signal into a sum of sinusoids as Fourier series expansion and to the sinusoids as "harmonics" of the signal. The harmonics are indexed by an integer (1st, 2nd, 3rd, 4th, etc), which is also the number of cycles the corresponding sinusoids make in the interval T. In other words, the harmonics are spaced by $1/T$ Hz. Fig. 3b shows an example of the harmonics of a periodic signal with $T = 0.1$ s.

Let's consider as second example a simple square wave $x(t)$ with fundamental period $T = 1$ s, amplitude 0 or 1 with duty cycle 50 % (that means that in each period T, the waveform will be equal to 1 for half of the time and equal to 0 otherwise) (Fig. 4a).

This signal can be expanded as a sum of an infinite number of sinusoids, with frequencies $1/T$, $2/T$, $3/T$, etc. Fig. 4b shows some of the sinusoidal components the signal can be expanded into. The first component (orange sinusoid) has the same period (and thus frequency) as the original signal, and it is named "fundamental harmonic". The second component has zero amplitude and the third (purple sinusoid) has a period equal to 1/3 of the original signals and thus varies at 3 times faster frequency, and it is named "third harmonic." The fourth component has zero amplitude and the fifth (red sinusoid) is the "fifth harmonic," and so on. In this specific case, the "even harmonics" (the second, fourth, and so on) are not needed to synthetize the signal, but this is just a specific case.

Fig. 4c and d show how summing up more and more components depicted in Fig. 4b (synthesis), the resulting signal resembles better and better the original signal. If we consider only a finite number of harmonics, the reconstructed signal is just an approximation of the original one. Adding up all harmonics (that, in the provided example, are infinite) would allow perfect reconstruction.

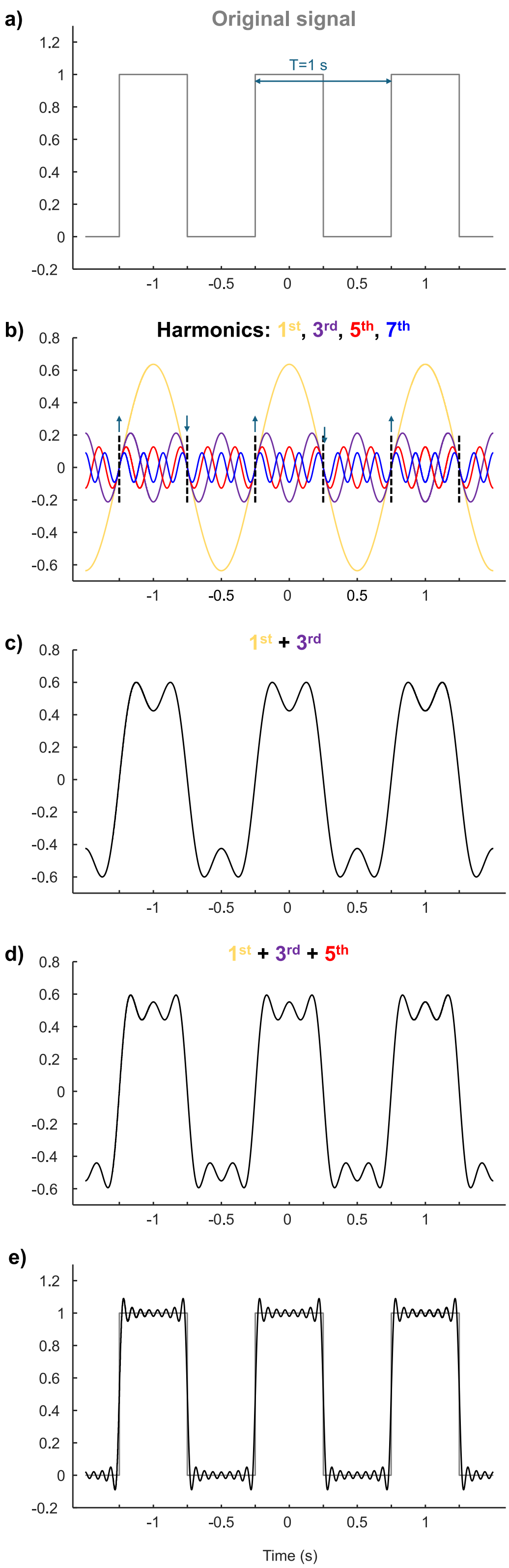

Fig. 4. a) A square wave with amplitude 1 V and period $T = 1$ s. b) The first harmonics of odd order that can be used to synthetize the signal in a). c) An approximation of the signal in a) obtained by a weighted average of the first and third harmonics. The second and fourth harmonics have zero amplitude. d) The signal approximation improves if the fifth harmonic is also considered. e) Approximation obtained including the DC component and the harmonics up to the 15th.

Two features are noteworthy. First, the amplitudes of the harmonics are different. Therefore, the signal $x(t)$ is expanded as a weighted sum of the harmonics. Second, adding more high-frequency components helps to recreate the sharp edges in the original waveform whose amplitude instantaneously changes from 0 to 1 and vice versa. This reinforces the statement that high frequencies are related to rapid changes in a signal, as mentioned in section 3. In the case at hand, an infinite number of harmonics would be needed to synthesize the exact original signal, because of the discontinuity (instantaneous jumps from 0 to 1 and vice versa, black dashed lines). However, in this specific case, the first five harmonics (Fig. 4d) already provide a relatively good approximation of $x(t)$, that portends that, in this case, the high-order harmonics will have smaller amplitudes.

All the harmonics have an average value over their respective period equal to zero. Their sum has also an average value equal to zero over the period of the original signal $x(t)$, but $x(t)$ has an average value equal to 0.5. Therefore, to accurately reconstruct $x(t)$, we have to add 0.5 to the weighted sum of the harmonics, resulting in Fig. 4e. Using terminology borrowed from electronics, this constant value is called a "direct current" (DC) component or signal offset or bias.

4.3. Amplitude, power, and phase spectra

Each of the harmonics can be identified by 3 parameters, amplitude, frequency and phase. Usually, this is represented in two graphs that show the absolute value of the amplitude (Fig. 5a), and the phase (Fig. 5b) of each harmonic versus frequency. These two graphs are named amplitude (or magnitude) and phase spectra of the signal $x(t)$, or, taken together, the Fourier spectrum of $x(t)$.

Both graphs (amplitude and phase spectra) are stem plots. Each stem (vertical bar) in the amplitude spectrum represents the amplitude of each harmonic at the corresponding frequency. For instance, for the first harmonic ($f = 1$ Hz), the amplitude is 0.637, as for the orange sinusoid; for the third harmonic ($f = 3$ Hz), the amplitude is 0.212, as for the purple sinusoid; and so on. The stem has zero amplitude for the frequencies corresponding to the even harmonics because they are not necessary to synthetize the signal being analysed. Because the harmonics are spaced by $1/T$, this is the "frequency resolution" (1 Hz) of the spectrum. That means that longer periods correspond to higher frequency resolutions, that is more closely spaced harmonics.

For $f = 0$ Hz, the amplitude is 0.5 which is the average value of the signal $x(t)$ over a period. In line with the other components in the

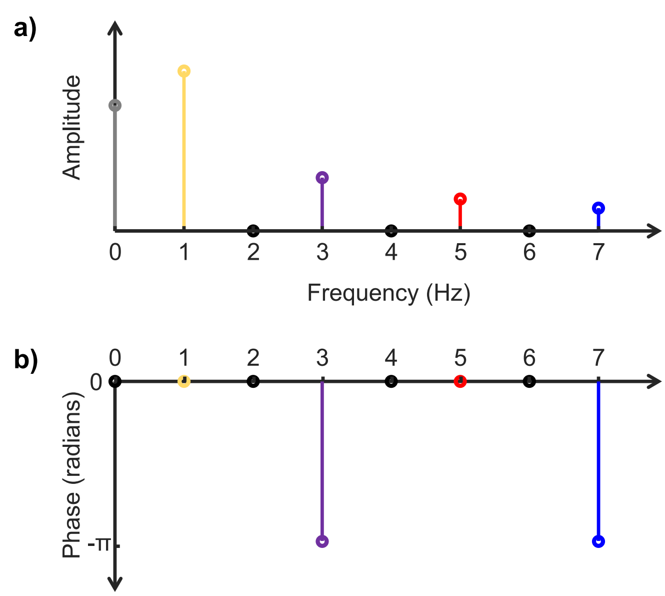

Fig. 5. a) Amplitude and b) phase spectra of the signal represented in Fig. 4a.

amplitude spectrum, you can consider the offset (DC component) as the amplitude of a sinusoid oscillating at the respective frequency (0 Hz), and, therefore, a constant value.

A similar reasoning applies to the phase spectrum (Fig. 5b). For a series of cosine functions, the phase is $-\pi$ radians for the harmonics of odd order and zero otherwise. That means that $x(t)$ can be synthetized as

$$x(t) = 0.5 + 0.637\cos(2\pi t) + 0.212\cos(6\pi t - \pi) + 0.127\cos(10\pi t) + 0.091\cos(14\pi t - \pi)\cdots \tag{4}$$

The coefficients of this linear combination (the weights of the sum) are named Fourier series coefficients and correspond to the amplitude of the sinusoidal components.

The relationship between the amplitude spectrum and the waveform $x(t)$ is easy to grasp. Amplitudes at different frequencies represent how strong is the sinusoidal component having that frequency in the original signal. The relationship between the phase spectrum and the waveform $x(t)$ may be less intuitive, but the phase spectrum plays an equally important role in the waveform shaping. We can observe in Fig. 4b that all sinusoidal components have negative values before and positive values after the rising edge of each square wave (see arrow up). This can only be achieved by shifting some of the sinusoids in time, according to their phase, and gives rise to a steep increase in the amplitude. On the other hand, all sinusoidal components have positive values before and negative values after the falling edge of each square wave (see arrow down), which gives rise to a steep decrease in their sum. This example highlights that to synthesize a specific signal we need to properly combine amplitude and phase of the different sinusoids. The explanation of the role of the phase has been reported for the sake of completeness, but in the reminder of the tutorial, we will focus on the amplitude spectrum because more relevant for the sEMG frequency analysis.

The square wave being analysed can be synthetised using only co-sines. This holds true for all signals that are symmetric about the vertical axis. In general, both cosines and sines are needed and in the most general case, indicated with A_{cn} and A_{sn} the coefficients of the cos and sin terms for the nth harmonic, the corresponding stem in the magnitude spectrum will have amplitude $\sqrt{A_{cn}{}^2 + A_{sn}{}^2}$. In general, the Fourier transform of a signal $x(t)$ is a complex number $X(f)$ and $\sqrt{A_{cn}{}^2 + A_{sn}{}^2}$ represents its absolute value or magnitude. Therefore, the amplitude spectrum is also indicated as $|X(f)|$, where the symbol $|\bullet|$ means magnitude.

The square of the amplitudes of the harmonics provides the power spectrum (or auto-spectrum) of the signal, that is $|P_{xx}(f)| = |X(f)|\bullet|X(f)| = |X(f)|^2$, which shows how the power of the signal is distributed among the harmonics. For this reason, $|P_{xx}(f)|$ is often called “power spectral density” (PSD). The unit of the PSD is the square of the unit of the amplitude value per Hz; e.g., if the time signal has amplitude in V, the PDS unit is V^2/Hz. The average power in a signal over a certain frequency range can be calculated by integrating the PSD over that frequency range.

A smoother estimate of $|X(f)|^2$ is obtained by averaging the power spectra (magnitudes squared or power of the harmonics, or PSD) over a number of epochs of equal duration (possibly partially overlapping). This approach is called the “Welch periodogram”, and it is used with noisy data because the averaging procedure reduce the variance of the estimator. As a result, the frequency resolution decreases due to the shorter epochs compared to the full-sized signal. This procedure is described in Fig. 6 and is implemented in commercial software.

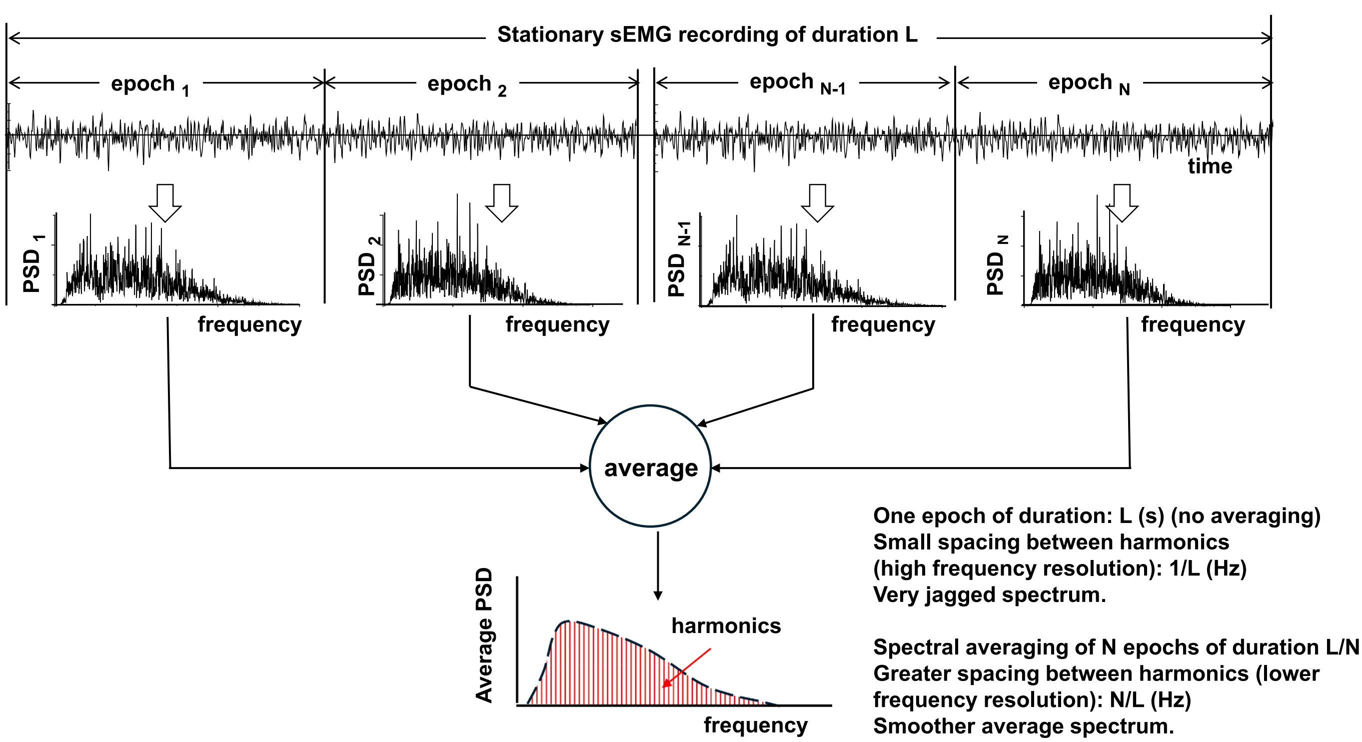

Fig. 6. Average of N power spectra. Consider a long random-like signal recording (e.g., sEMG) divided into shorter recordings of L s duration (e.g., 2 s). The harmonics will be spaced by $1/L$ Hz (e.g., 0.5 Hz). The magnitude squared of each harmonic provides the power spectral density (PSD, power of each harmonic) that will be very jagged. One way to make this spectrum smoother is to divide the L s recording into N epochs of duration L/N s (e.g., $N = 5$, that is epoch duration = 0.4 s) and compute the N PSDs of the N epochs with a frequency resolution of N/L Hz (e.g., 2.5 Hz) and average the N values of each harmonic amplitude. The spectrum will be smoother but with a poorer frequency resolution that may still be acceptable. The epochs may overlap (e.g., 50 %). In this case $N_{over} = 2N - 1$ (e.g., 9 epochs) and the PSD will be even smoother but with the same frequency resolution. This is called the “Welch periodogram” and is an option available in most programs for sEMG spectral analysis. Spectral features will be estimated every L s with lower random variations (Farina and Merletti, 2000). Modified from https://www.robertomerletti.it/en/emg/material/teaching/module4.

4.4. The discrete Fourier transform of non-periodic signals

The Fourier series is a nice tool for analysing periodic continuous-time signals, that are deterministic. A deterministic signal is a signal that can be defined exactly by a mathematical formula. However, the signals of our interest (sEMG) are not periodic. Also, the sEMG signals we analyse are discrete since they are recorded after analog-to-digital conversion (Merletti and Cerone, 2020). Additionally, the surface EMG signal is random, i.e., not deterministic. So, how do we transform the signals in the frequency domain in that case?

We use a mathematical "trick" consisting in taking a segment of the sEMG signal called "epoch" or "time window" of duration T s and assuming that it repeats periodically with period T. Although this is not true, it enables us to determine the Fourier spectrum as described in sections 4.2 and 4.3. For deterministic signals, the Fourier transform provides the actual spectrum. For random signals, a transformation to the frequency domain provides an estimation of the spectrum. The spectrum will have a frequency resolution of $1/T$, therefore, the choice of the epoch length determines the spectral resolution (spacing between the harmonics). To have high resolution (small distance between the stems in the spectrum), T should be long. However, the epoch should also be short enough so that the signal is reasonably "stationary" during each epoch, that is its time and frequency features may be assumed to remain constant during each epoch (section 6). Therefore, the choice is a trade-off of these two opposing requirements and depends on how fast the sEMG is changing due to the velocity of shortening or lengthening of the muscle, changes in neural input, and other factors.

If the first and last signal values or samples of the epoch are very different, high frequency components are introduced because of the discontinuity when the epoch is made periodic. To avoid this effect the signal is often multiplied by a trapezoidal function that forces the values to go to zero at the beginning and end of the epoch. This function is called a "window" and may have different shapes (e.g., half period of a sine). Although commercial software provides a selection of different windows, the effect of "windowing" the raw sEMG on the shape of its amplitude or power spectra is small and will not be further discussed here.

We will be back to the concept of epoch selection is section 6. Regardless of the chosen epoch duration, the time features of the signal in subsequent epochs will never be identical (due to the random nature of the signal) and will show fluctuations from epoch to epoch. To reduce these fluctuations, the spectrum can be estimated by averaging the spectra obtained for a number of sub-epochs (Fig. 6). The epochs may be partially overlapping to **increase their number and get a more reliable average.**

When analysing EMG signals, apart from the absence of periodicity, there is another difference compared to when we have presented the Fourier series. Sampled sEMG signals are discrete, rather than continuous in time. Without entering the mathematical explanation, if the periodic signal we want to transform to the frequency domain is sampled with sampling period T_{samp} (that is the distance between two samples), the spectrum is periodic with period equal to $1/T_{samp}$. Also, if N is the number of samples in a period ($T = N \bullet T_{samp}$) of the discrete-time signal, the number of samples in a period of the spectrum ($1/T_{samp}$) is also N (the stems in the frequency spectrum are spaced by $1/T$). Therefore, it is enough to calculate N values of the transform. The transform of a periodic discrete-time signal is known as Discrete Fourier Transform (DFT). Actually, the spectrum is also symmetric[1] with respect to the $f = 0$ Hz value and therefore it is sufficient to calculate $N/2$ coefficients. This is why in the power spectrum representation, the frequency axis spans the interval from 0 to half of the sampling frequency. This implies that there are mirror-like harmonics at negative frequencies: this is a purely mathematical concept that is not relevant for our purposes in this tutorial.

Since the frequency resolution is equal to $1/T$, it is possible to artificially increase T by adding samples equal to zero (zero padding) so that the number of samples becomes $N' > N$, the period duration becomes $T' > T$, and the spacing between spectral lines becomes $1/T' < 1/T$. This improvement in the resolution is purely "cosmetic" and does not increase the information. Zero padding is applied after "windowing" the raw sEMG, in order not to add discontinuities.

So far, we have focused on the meaning of the Fourier spectrum, but how do we actually calculate it? Consistently to our choice to limit the mathematics presented in the tutorials, we do not report the expressions to calculate the coefficients of the Fourier series and the DFT. The interested reader is referred to textbooks, teaching modules, tutorials and consensus papers on this topic.

Most software commonly used for sEMG analysis (e.g., Matlab, Python, or specific programs) have built-in functions to calculate the DFT. The most widely used algorithm is known as Fast Fourier Transform (FFT) (Cooley and Tukey, 1965), which requires a number of samples equal to a power of 2. Typically, zero padding is applied before the FFT, so that the total number of samples in an epoch N' is equal to the first power of 2 greater than N.

5. EMG frequency content

In section 4, we described how we can map a signal from the time domain to a frequency spectrum through the Fourier transform. In section 5, we aim to understand when and why it is useful to apply the Fourier transform to the sEMG signal and interpret its PSD.

5.1. EMG signal bandwidth

For some signals we know in which range of frequencies we expect the signal to have spectral components. For instance, we know that the surface EMG signal, the ECG, and the EEG have frequency components in the range 5 to 500 Hz, 0.5 to 150 Hz, and 1 to 80 Hz, respectively. We use the term "bandwidth" to indicate the width of that frequency range. In other words, the bandwidth is the difference between the maximal and the minimal frequency of the signal's harmonics. For instance, we can notice in Fig. 6 that the amplitude of the spectrum is negligible outside the EMG bandwidth. The actual bandwidth depends on the muscle type, on the adipose tissue between the muscle and the electrode, on the electrode position and size, on the detection modality (monopolar vs bipolar), and on the center-to-center interelectrode distance (in case of bipolar detection) (De Luca et al., 2010; Merletti and Muceli, 2019).

The thicker the fat layer or the deeper the MUs, the smoother the EMG signal and the narrower its bandwidth. The bottom panel of Fig. 7 shows two MUAPs detected from the biceps brachii of a healthy subject with intramuscular EMG (that is thin wires placed within the muscle). The upper panel shows the same action potentials as detected with sEMG. We can observe that the intramuscularly detected action potentials have a relatively shorter time support (time interval where the amplitude is different from the background noise) compared to the corresponding action potentials detected at the surface of the skin. The amplitude of the intramuscular action potentials changes rapidly (they are spiky), while the sharp peaks are strongly attenuated in the surface action potentials (they are smoother). Smooth changes are characterized by low frequency components, while rapid changes correspond to high frequency components, as stated in section 3. Therefore, the bandwidth of the intramuscular EMG extends beyond the bandwidth of the surface EMG signals, at least until 1500 Hz. The bandwidth upper limit depends on the detection system (e.g., needle vs fine wires).

The bandwidth of sEMG is generally greater in case of sensor placement on top of the innervation zone or on the junction between muscle and tendon (De Luca et al., 2010). Considering electrode size, bigger electrodes smooth the signal compared to smaller electrodes (see

[1] The symmetry is due to the fact that the signal is real. The explanation goes beyond the scope of this tutorial.

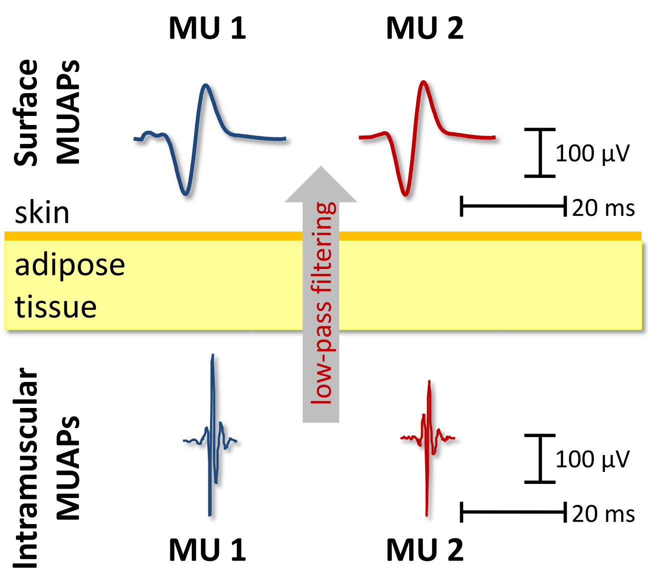

Fig. 7. Effect of the volume conductor. Two motor unit action potentials (MUAPs) are detected with fine wires from the biceps brachii muscle and high-pass filtered to facilitate decomposition. The two intramuscular action potentials are spiky and have a short time support. The volume conductor constituted by the muscle tissue, adipose tissue, and skin layers acts as a low-pass filter, making the surface action potentials smoother and broader. Reproduced from (Farina and Holobar, 2016), with permission.

Fig. 12 in (Merletti and Muceli, 2019)) because they average it over the contact area.

When do we make use of the notion of bandwidth in the detection and processing of EMG signals? Mainly a) to understand how fast to sample the signal, b) to filter it, as detailed in section 5.2 and section 5.3 and c) to detect physiological changes that affect the sEMG spectrum, as detailed in section 7.

5.2. Sampling theorem

Sampling is the process that converts a continuous-time signal into a discrete-time signal (Fig. 1b) so that it can be processed by a computer. In section 2, we anticipated that sampling has to be sufficiently fast not to lose or distort the information carried out by the continuous-time signal. But, what does "sufficiently fast" mean? According to the Nyquist theorem, the sampling rate must be greater than twice the signal's bandwidth to allow recovery of the entire information contained in the continuous-time signal. Sampling at 3–4 times the signal bandwidth is better because the reconstruction of the time-continuous signal from its samples is actually better. In fact, the theoretical limit of just more than twice the bandwidth requires the use of an ideal filter in the reconstruction process, which cannot be easily achieved in practice. This is why, the surface EMG signal is often sampled at atleast 1 kHz or (and better) at 2 kHz and the intramuscular EMG signal at higher frequency (e.g., 10 kHz).

5.3. Filtering

When we acquire EMG signals, we always record noise mostly generated at the electrode–skin interface. We refer generically to noise to indicate an unwanted random signal. Periodic or quasi-periodic undesired signals are referred to as interferences (e.g., power line or ECG interference). Noise and artifacts may come from different sources. For instance, most of the devices used to acquire EMG signals are tethered and the cables that connect the electrodes to the acquisition device may move during acquisition, pulling on the electrodes and causing transients of the electrode–skin interface voltage known as motion artifacts.

The power line may introduce an interference (PLI), that consists in a signal at 50 or 60 Hz, often presenting harmonics at multiples of these frequencies, superimposed to the EMG signal. This interference may be difficult to detect in the time domain signal. However, it can easily be noticed in the magnitude or power spectra of the acquired signal as a line at 50/60 Hz. This example clearly shows how beneficial can be to change the signal "description" by passing from the time to the frequency domain.

Noise or interferences may also be due to electrical instrumentation present in the room(s) near to the EMG acquisition place. Since noise, artifacts, and interferences are unwanted components, how can we avoid them? The best option is to try to eliminate these sources prior to the acquisition. Proper skin treatment is the first step towards the acquisition of a noise and artifact-free signal. Rubbing the skin with abrasive paste has been shown to result in less noise compared to rubbing the skin with alcohol or not treating the skin at all (Piervirgili, Petracca and Merletti, 2014). Electrodes must be well secured to the skin, e.g., by applying elastic bands or adhesive tape. Cables must be fixed well to avoid their movements during the recordings. If multiple instruments are used, they should all be connected to the same power outlet and ground to reduce PLI. Other requirements for the instrumentation are summarized in Table 2 of (Merletti and Cerone, 2020).

Despite these precautions, sometimes noise or interferences cannot be avoided. If this is the case, is there anything we can do once the signal has been recorded to eliminate noise? In general, all frequency components outside the sEMG bandwidth should be eliminated as they do not belong to the sEMG signal. This can often be achieved by filtering the signal. A filter is a system that attenuates specific harmonics. A filter that attenuates the high frequency harmonics is known as "low-pass" because low frequencies are retained. This filter is typically used to eliminate high frequency noise. On the contrary, a "high-pass" filter attenuates the low frequency harmonics and preserves the high frequencies. This type of filter is used to eliminate the wandering of the sEMG signal baseline due to cable motion and to eliminate any DC component so that the filtered signal has zero mean. A filter that maintains only the frequencies within a set bandwidth is referred to as "band-pass." A "notch" filter selectively eliminates a specific frequency. The notch filter is often used to eliminate the PLI. In that case, a notch width of 2–3 Hz is recommended because the line frequency may fluctuate within ± 1 Hz. However, the PLI is within the sEMG bandwidth, therefore the notch filter removes also spectral lines of the sEMG. Spectral interpolation may be more suitable because it preserves the 50/60 Hz component of the sEMG signal (Merletti and Cerone, 2020). More sophisticated techniques are required to reduce ECG interference when the sEMG is detected from chest muscles (Mak, Hu and Luk, 2010).

Low-pass filtering is also very important in smoothing the rectified sEMG as discussed in Figs. 5 and 6 of the tutorial on amplitude estimation (Clancy et al., 2023).

5.4. Cross-spectrum and coherence

Each harmonic of the Fourier transform of a signal is a sinusoid with an amplitude (magnitude) and a phase providing the magnitude and phase spectra (section 4.3). Consider now two different signals, $x[n]$ and $y[n]$ having power spectra $P_{xx}(f)$ and $P_{yy}(f)$. Some of their harmonics may show similar fluctuations of their power in time, that is, they may be "correlated". This correlation may be stronger for some harmonics or frequency bands and weaker or absent for others. This correlation may be quantified and described in the frequency domain using the concept of cross-power spectrum defined as $|P_{xy}(f)| = |X(f)| \bullet |Y(f)|$, where the magnitude of each Fourier transform, $|X(f)|$ and $|Y(f)|$ must be computed as the average of the magnitudes estimated over a number of sub-epochs of equal duration with the same overlapping (Welch periodogram). The cross-spectrum $P_{xy}(f)$ has a magnitude and a phase just as $X(f)$ and $Y(f)$. Here, we consider only its magnitude $|P_{xy}(f)|$. If the two signals $x[n]$ and $y[n]$ are not correlated (e.g., $y[n]$ is a process not derived from $x[n]$), their cross-spectrum is zero, that is, $|P_{xy}(f)| = 0$ for all frequency values (all harmonics). If $|P_{xy}(f)| \neq 0$ for some harmonics, then $x[n]$ and $y[n]$ show similar fluctuations in time indicating the presence of "coherence" between the two signals in the frequency domain. This means that the amplitudes of some harmonics of the two signals are fluctuating in a correlated manner.

To clearly outline such a degree of common fluctuation and make it independent of the amplitudes of the harmonics, that is express as a percentage of the amplitude of each harmonic, a normalization is made to show the "Magnitude-Squared Coherence" (MSC) between the two signals defined as

$$MSC(f) = \gamma^2(f) = \frac{|P_{xy}(f)|^2}{P_{xx}(f)P_{yy}(f)} \quad (5)$$

This expression may seem complicated but built-in functions are available in some software (e.g., in Matlab). To properly use those functions it is important to understand the meaning of parameters such as the epoch length, the overlap between epochs, the zero padding, that are requested as input of the functions.

$MSC(f)$ is an index of coherence between $x[n]$ and $y[n]$ and, for each value of f, takes values between 0 and 1, where 0 means uncorrelated fluctuations (0 % correlation, no coherence) and 1 indicates fully correlated fluctuations (100 % correlation, full coherence) between corresponding harmonics of the two signals. Two examples of MSC between two EMG signals from two muscles of a patient with pathological tremor are shown in Fig. 8. The coherence peaks at 5–6 Hz indicate that the two muscles have similar activation signals in the tremor band. MSC has also been used to assess functional connectivity (i.e., correlated input to spinal motor neurons) in the motor system at multiple frequency bands (Kerkman et al., 2018).

To determine in which frequency range the levels of coherence are significant, the confidence level (CL) of the coherence profile is calculated by the following equation (Rosenberg et al., 1989):

$$CL = 1 - (1 - \alpha)^{\frac{1}{N_{epoch}-1}} \quad (6)$$

where N_{epoch} is the number of epochs and α is a statistical thereshold. For instance, if α is set to 0.95, values of coherence are considered significant when they exceed the 95 % CL. Since the coherence is derived from the auto- and cross-spectra, that can be only estimated given the random nature of the sEMG signal, the introduction of the CL is necessary to provide a value to distinguish the random fluctuations of the coherence

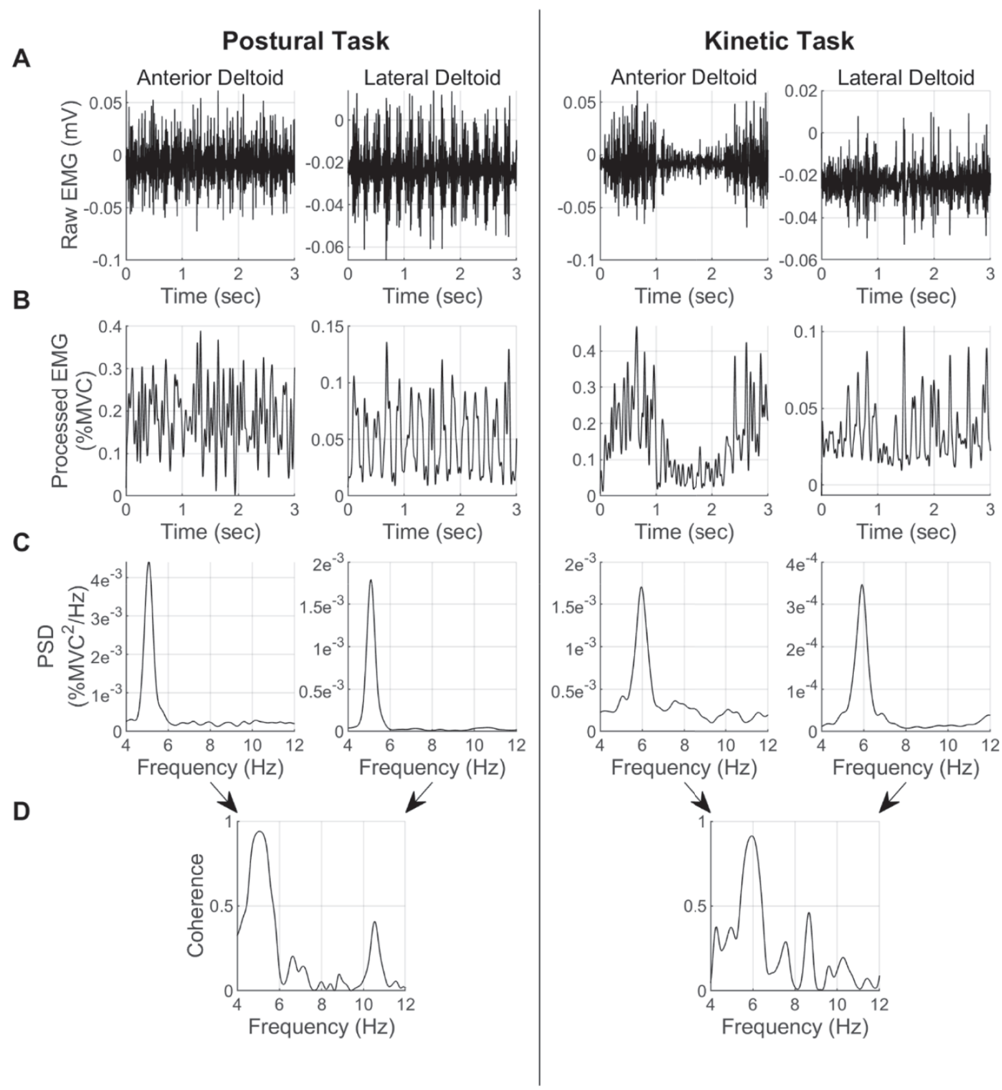

Fig. 8. A) Raw and B) rectified and smoothed EMG recorded from the anterior and later deltoid of a patient with essential tremor. C) The corresponding power spectral density and D) coherence between the activity of the two muscles. The coherence plot shows common fluctuations at 5 Hz in the postural task and 6 Hz in the kinetic task, related to shared tremorogenic muscle activity. Figure reproduced from (Free et al., 2023).

estimation from zero.

It is important to notice that the existence of correlation or coherence between two signals does not necessarily mean that one signal is causing the other. They may have common components because they share a common third signal or they may be in part "driven" by a third signal. However, if one signal is a filtered version of the other, their MSC is 1 because normalized fluctuations of the harmonics' amplitudes are maintained through filtering.

These concepts have important applications in the study of the EEG and sEMG signals since two EEG, two sEMG, or an EEG and a sEMG signal may show coherence. This implies that there is some link (causal or non-causal) between them, and coherence indicates how strong and at which frequencies such link is present. This is an important and useful tool for the study of the neuromuscular system (Grosse, Cassidy and Brown, 2002; Kerkman et al., 2018; Free et al., 2023) that will not be discussed in this tutorial.

6. Signal stationarity

Signal processing textbooks indicate that the Fourier transform is meaningful only for "stationary" signals, that is for signals whose amplitude and spectral features do not show trends or large variations in time. Because of multiple phenomena including muscle fatigue, MU rotation/substitution, dynamic contractions, and changes in task control strategy (even under isometric contractions), the resulting sEMG is a non-stationary signal and this concept deserves clarification. Fig. 9 illustrates this concept using a simple example based on a deterministic signal of 2 s duration. This signal is the combination of two sinewaves: $s_1(t) = A_1 sin(2\pi f_1 t)$ having amplitude $A_1 = 1$ V and frequency $f_1 = 10$ Hz whose amplitude spectrum is a single line at 10 Hz, and $s_2(t) = A_2 sin(2\pi f_2 t)$ having amplitude $A_2 = 1.5$ V and frequency $f_2 = 5$ Hz whose amplitude spectrum is a single line at 5 Hz. In Fig. 9a, $s_1(t)$ is present for the first second and $s_2(t)$ is present for the second second of a 2-s epoch. The resulting signal is obviously non-stationary because of the sudden change at $t = 1$ s. If we compute its Fourier transform using a 2-s epoch we obtain the harmonics indicated in Fig. 9b. They do not indicate when each signal is present. Fig. 9c depicts a signal $s_3(t) = s_1(t) + s_2(t)$, which is clearly stationary over the 2-s epoch. Its Fourier transform includes the harmonics indicated in Fig. 9d. In both cases (Fig. 9b and c), the harmonics are spaced 0.5 Hz apart because the epoch duration is 2 s. The two signals in Fig. 9a and c are very different but have similar spectra showing large harmonics at 5 Hz and 10 Hz and ambiguity is present (the explanation of presence of other harmonics in Fig. 9b, due to the 0.5 Hz periodicity of the signal, exceeds the purpose of this tutorial). The choice of four non-overlapping epochs of 0.5 s each would have shown the changing spectrum from epoch 2 to 3 in the case depicted in Fig. 9a. A similar reasoning can be applied to the analysis of the sEMG signal.

6.1. Epoch duration selection

Non-stationarities, due to muscle fatigue and/or MU recruitment/derecruiment, are masked if long epochs are used and become apparent only if shorter epochs are chosen. A signal whose spectrum changes slowly from epoch to epoch (like the sEMG) is called "quasi-stationary". So, which epoch duration should be selected for the frequency analysis of sEMG?

At high contraction levels (>50 % of the maximal voluntary contraction (MVC)), all or most MUs are recruited, the muscle is ischemic and the intramuscular pH decreases causing a rapid decrease of the muscle fiber conduction velocity (MFCV) and consequent widening of the MUAPs resulting in spectral narrowing (compression) (Fig. 10).

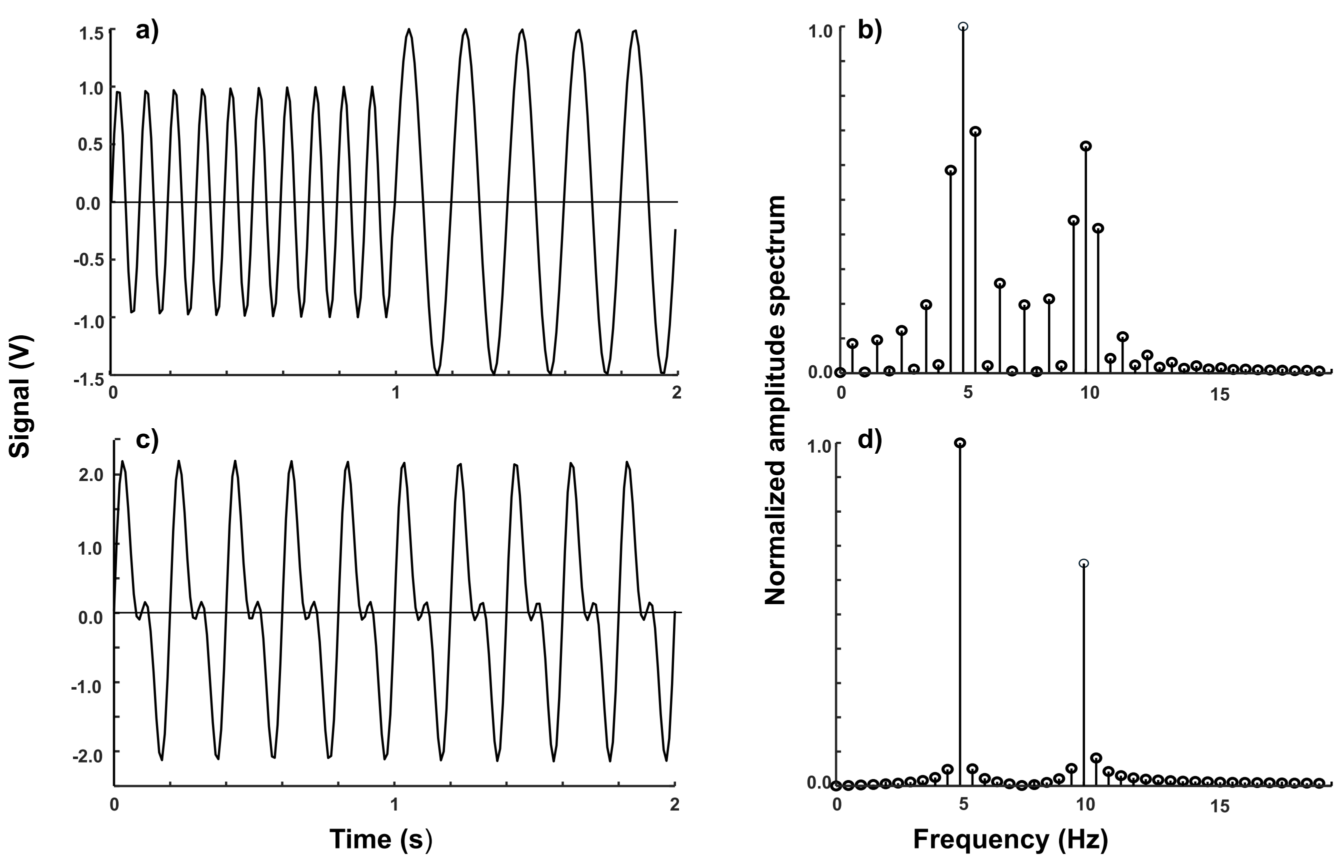

Fig. 9. Frequency analysis of stationary and non-stationary signals. a) Example of a 2-s epoch of a non-stationary signal whose Fourier transform is depicted in b). c) Example of a 2-s epoch of a stationary signal whose Fourier transform is depicted in d). Fourier transforms are similar and normalized with respect to the respective peak values. See text for explanation.

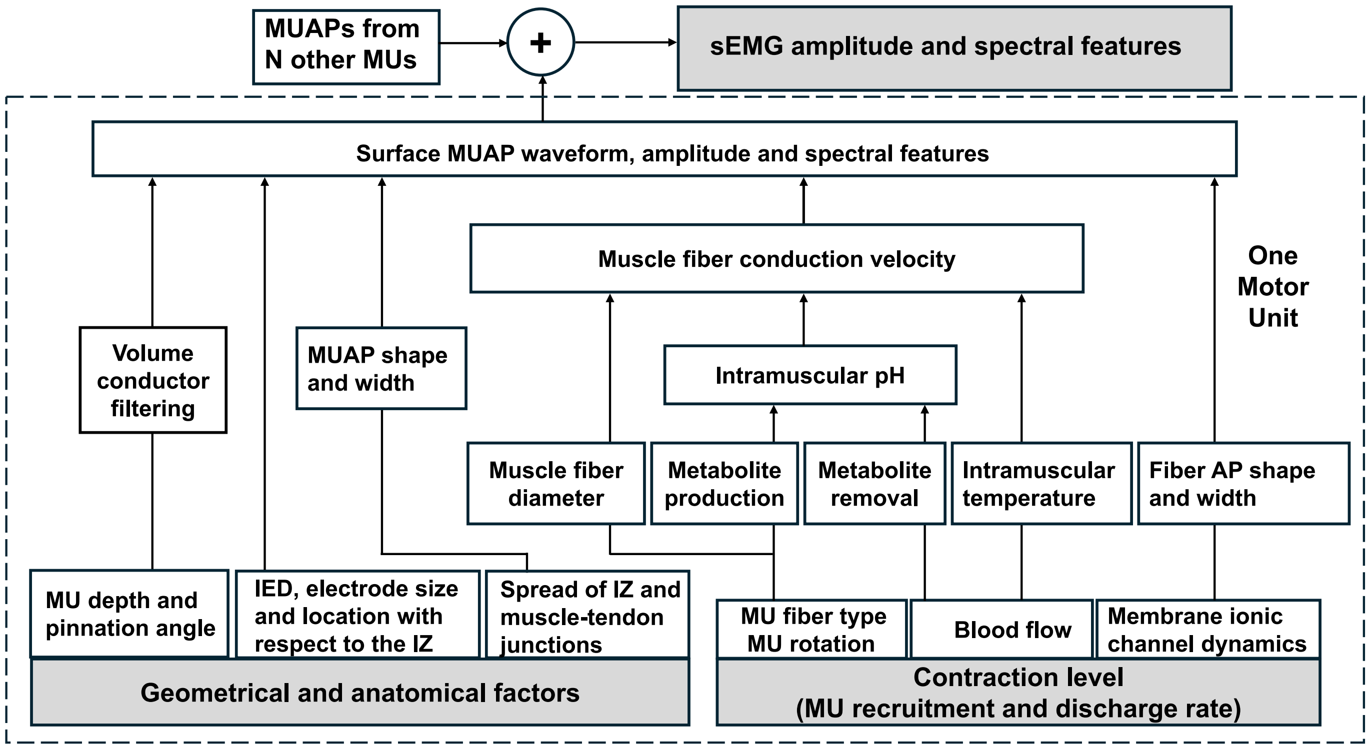

Fig. 10. Geometrical and physiological factors determining a surface motor unit action potential (MUAP) waveform, and amplitude and spectral features of a surface EMG signal. Many MUAP trains add up algebraically on the skin surface to produce the sEMG signal. MU: motor unit; IED: inter-electrode-distance; IZ: innervation zone.

This results in a non-stationary sEMG, that requires short epochs for tracking these changes. Short epochs result in poor spectral resolution (because of the wide separation between harmonics), and thus poor estimates of the spectral features (see section 7). (Farina and Merletti, 2000) found that epochs ranging from 0.125 s to 0.5 s with 50 % overlap (see their section 3.2) provide an acceptable trade-off between these two needs. These epochs correspond to spacings between harmonics in the range of 8–2 Hz, respectively. For lower-level voluntary contractions (more stationary signals), epoch durations of 0.5–1 s are acceptable.

7. Which information can we gather from the surface EMG spectrum?

7.1. Anatomical and physiological correlates

The sEMG is often considered a “difficult to analyse” signal because each of its features is affected by many anatomical/geometrical and physiological factors (Farina, Cescon and Merletti, 2002). Fig. 10 depicts some of these factors as they affect each MUAP and, therefore, the sEMG. The most important physiological factor is the MFCV which is mainly affected by muscle fiber size, intramuscular pH and temperature (Stulen and De Luca, 1981; De Luca, 1984; Arendt-Nielsen and Mills, 1985; Andreassen and Arendt-Nielsen, 1987; Arendt-Nielsen et al., 1989; Brody et al., 1991). A decrease of MFCV causes a widening of the detected MUAP and therefore a narrowing of its spectrum (see slides 37–39 of https://www.robertomerletti.it/en/emg/material/teaching/module5). When this happens to many MUs the power spectrum of the sEMG becomes narrower, that is, it is “compressed” towards the lower frequencies because the amplitudes of the higher harmonics decrease and the amplitudes of the lower harmonics increase.

How can these spectral changes be quantified and described in a compact way by means of some index that would reflect the MMMF? Two main and similar spectral indices have been identified and extensively reported in the scientific and clinical literature: the median spectral frequency f_{median} (MDF) and the mean spectral frequency f_{mean} (MNF). The MDF is the frequency value that divides the sEMG power spectrum into two areas of equal power. In other words, the sum of the powers of all the harmonics below the MDF is equal to the sum of the powers of all the harmonics above it. The MNF is the centroid frequency of the sEMG power spectrum, that is the frequency of its center of gravity or barycenter. It is calculated as an average of the power lines of the PSD, weighted by the harmonic frequencies, divided by the sum of the power lines. In the very uncommon case of PSD symmetric with respect to some central spectral line, the MNF and the MDF coincide with such line. In general, the sEMG PSD is skewed and the MNF is greater than the MDF. Their percent difference ($100(MNF - MDF)/MNF$) or ratio (MNF/MDF) provides an index of spectral skewness. Since the sEMG is a random like signal, its spectral estimates have epoch to epoch fluctuations and MDF and MNF are estimates with a mean and a standard deviation even for stationary signals. Theoretical considerations and computer simulations show that the standard deviation of MDF across epochs is 25 % greater than that of MNF (Balestra, Knaflitz and Merletti, 1988). For this reason, MNF is usually preferred.

During sustained isometric constant force contractions, a decrease of MFCV results in a decrease of MNF and MDF. MFCV can be measured only in superficial fusiform muscles and may be overestimated because of the non-propagating “end-of-fiber effect” (see Fig. 4 of (Merletti and Muceli, 2019)). On the contrary, MNF or MDF can be estimated on any superficial muscle and sense the widening of the non-propagating “end-of-fiber effect”. Therefore, the assessment of spectral compression from the MNF or MDF may be a more viable and more sensitive option for estimation of MMMF during sustained constant force isometric contractions when other factors (Fig. 10) remain constant (Stulen and De Luca, 1981; Sadoyama, Masuda and Miyano, 1983; Lowery et al., 2000). During dynamic contractions the muscle moves under the electrodes and the relation between MFCV and MNF may be seriously disrupted (Falla, Graven-Nielsen and Farina, 2006). More advanced methods, such as time–frequency analysis may be more appropriate to assess MMMF in dynamic contractions. The description of these methods goes beyond the scope of this tutorial. The interested reader is referred to (Knaflitz and Bonato, 1999; Bonato et al., 2001).

7.2. sEMG frequency analysis: Applications

The most important application of sEMG frequency analysis is the monitoring of localized MMMF that anticipate mechanical contractile fatigue, that is the declining ability to sustain a required effort or task (De Luca, 1984; Basmajian and De Luca, 1985). Although the fatigue-related spectral compression of the sEMG was known earlier, the theory of frequency analysis of sEMG was developed by Lindstrom and Magnusson in the 70′s (Lindstrom and Magnusson, 1977) and applied in research by De Luca and others in the 80′s (Stulen and De Luca, 1981; Masuda, Miyano and Sadoyama, 1983; Sadoyama, Masuda and Miyano, 1983; De Luca, 1984; Arendt-Nielsen and Mills, 1985; Basmajian and De Luca, 1985; Andreassen and Arendt-Nielsen, 1987; Arendt-Nielsen et al., 1989). Important developments took place in the 90′s (Brody, 1991; DeAngelis et al., 1990; Merletti et al., 1990; Merletti and Roy, 1996) while clinical research applications developed in the new century (Larivière et al., 2008), as well as books and tutorials aimed at clinical users in various fields (Barbero, Merletti and Rainoldi, 2012; Butz, 2015; Vowels, Vowels and Wood, 2023). The literature concerning the interpretation of the sEMG PSD is very extensive, particularly in the fields of sport and ergonomics. Only a few fundamental references are reported here, and few examples are discussed.

Although the technique is often applied in dynamic conditions, sustained constant (or slowly varying) force isometric contractions provide the best test modality because the muscle is not moving under the electrodes and the signal is quasi-stationary within each epoch. This condition assures higher repeatability of the measurements (when they are repeated at different times to monitor changes) and provides a muscle "bench test", similar to the exercise ECG stress test, if geometric factors remain the same (Fig. 10) (Rainoldi et al., 1999, 2001; Falla et al., 2002). In these cases, a "fatigue plot" is a useful tool to display the MMMF during a sustained isometric constant force contraction. The fatigue plot shows the percent variations of MFCV, amplitude (average rectified value (ARV) or root-mean square (RMS)) and spectral (MNF or MDF) sEMG features with respect to their initial values, as indicated in Fig. 11 and in (Basmajian and De Luca, 1985; Merletti, Knaflitz and De Luca, 1990; Merletti and Roy, 1996; Merletti and Lo Conte, 1997; Rainoldi et al., 1999) and slides 48–53 of https://www.robertomerletti.it/en/emg/material/teaching/module7.

Fig. 11a and b indicate that similar individuals may show different fatigue plots possibly due to different distributions of muscle fiber types (an interpretation still to be verified in humans). Fig. 11c and d show the "ageing paradox" due to the selective loss of Type II fibers with age resulting in lower force but also lower MMMF. All panels show that MNF changes more than MFCV suggesting that MFCV is the main but not the only variable affecting sEMG spectral changes. The contraction levels (60 % MVC and 70 % MVC) imply that all or almost all the MUs are recruited, and MU rotation or substitution is absent.

Summing up, sEMG spectral measurements require great caution, competence and training in their execution and interpretation because of the many factors that may affect the results (Fig. 10) and the need for controlling that the geometric factors remain constant during a measurement and in subsequent measurements to be compared with the first.

8. Recommendations for best practices

The care and competence required to correctly extract useful

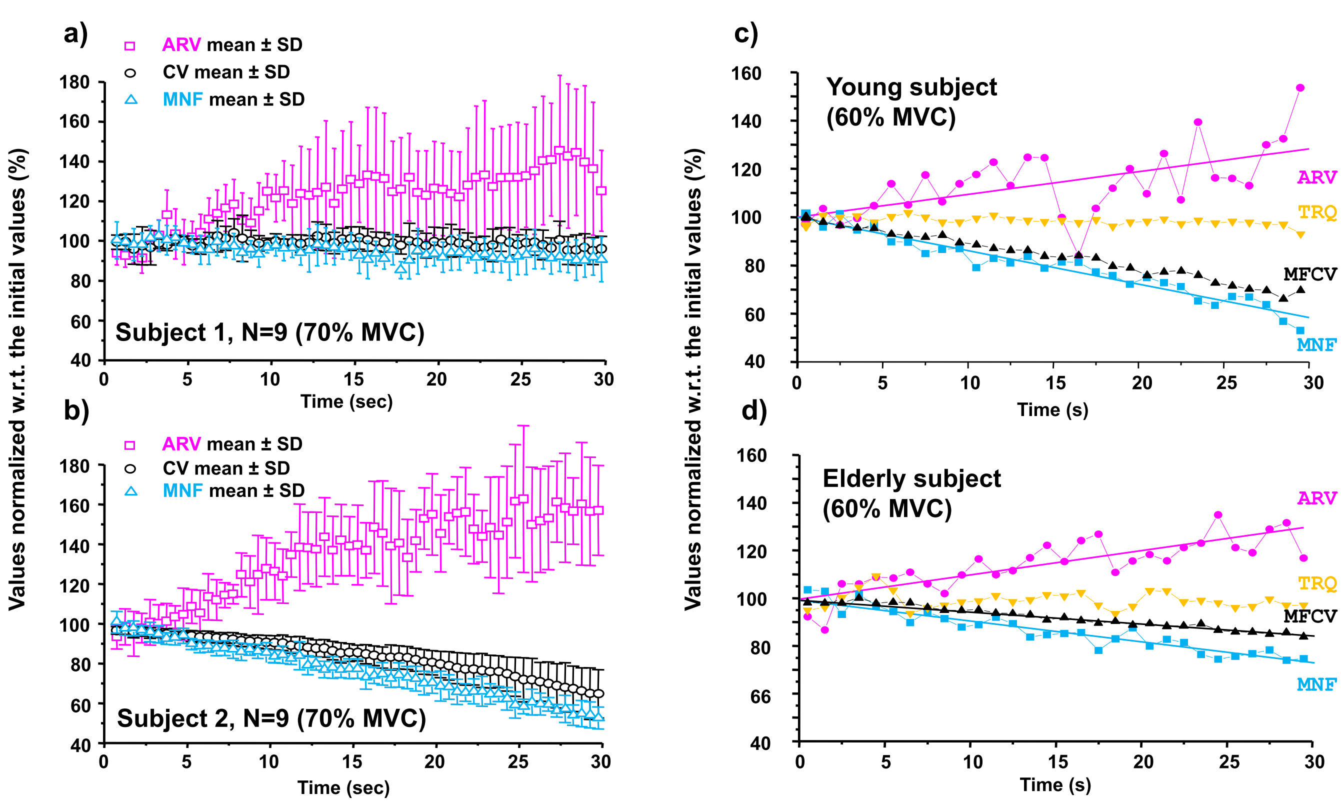

Fig. 11. Fatigue plots of the biceps brachii muscle in four subjects during isometric contractions sustained for 30 s. a), b) Two young males having similar age and lifestyle. Mean ± standard deviation of nine fatigue plots (three tests/day for three days), contraction level: 70 % MVC, epoch duration = 0.5 s; c), d) fatigue plots of a young (<30 year) and an elderly subject (>65 years), contraction level 60 % MVC, epoch duration = 1 s. ARV: average rectified value; MFCV: muscle fiber conduction velocity; MNF: mean spectral frequency; TRQ: torque at the elbow. See text for discussion. Panels a) and b) are modified from (Rainoldi et al., 1999) and panels c) and d) are modified from (Merletti et al., 2002), with permission.

Table 1
Checklist for designing a study, preparing/reviewing reports and manuscripts dealing with sEMG frequency analysis.

Topic	Item	Description of information to report	Reason for reporting, considerations and references
Tissue thickness	1	When possible, report the subcutaneous thickness measured by plicometry or ecography.	Amplitude and frequency features are affected by the subcutaneous tissue thickness.
Electrodes	1	Electrode size and inter-electrode distance	Electrode size and inter-electrode distance affect spectral features of sEMG (Farina, Cescon and Merletti, 2002; Merletti and Muceli, 2019; Besomi et al., 2024).
	2	Electrode location with respect to the muscle innervation zone or anatomical references. Electrode orientation with respect to the fiber direction.	When possible, the electrodes should be located between the innervation zone and one tendon ending (Merletti and Muceli, 2019).
Detection modality	1	Monopolar, single differential or other montages	sEMG spectral features depend on the detection modality.
Amplifiers and analog conditioning filters. Artefact reduction. Power line interference (PLI) prevention or reduction. ECG interference reduction.	1	Skin treatment, electrode and cable fixation	Skin treatment, electrode and cable fixations influence artifact and PLI.
	2	Type, order, and cut-off frequencies of the analog filters used for signal conditioning	If improperly selected, these cut-off frequencies affect the spectrum of the sEMG (DeAngelis et al., 1990).
	3	Type, order, and cut-off frequencies of the analog or digital notch filters for removal of PLI harmonics	Reduction of PLI at 50 Hz or 60 Hz may not be sufficient if PLI harmonics are present (Merletti and Cerone, 2020). Notch width of 2–3 Hz is recommended since the line frequency may fluctuate within ± 1 Hz. Spectral interpolation may be a better option as it preserves the 50 Hz component of the sEMG signal.
	4	Methods used for reduction of ECG interference, if relevant	ECG interference is often affecting sEMG from chest and back muscles.
Task and contraction modality.	1	Device used for locking the joint and measuring force or torque. Criteria adopted for defining MVC. Contraction levels in %MVC. Display of the target level for biofeedback.	Constant force or torque at the joint may not mean constant force of activated individual muscles. Variable load sharing among synergic/antagonist muscles may be present. Measurement of sEMG features of multiple agonist/antagonist muscle is desirable. Reporting the error in maintaining the target may be of interest.
	2	Isometric variable force contraction. Display of the target level for biofeedback.Rate of change of force or torque.	Reporting the root mean square error in tracking the target may be of interest.
	3	Contraction type, such as isometric constant force or variable force, dynamic contractions, isokinetic contractions, explosive contractions. Angle range and angular velocity of the movement. Force or torque level.	Measurements of sEMG features are very critical in non-isometric conditions since the muscle is moving under the electrodes. Electrode grids or linear arrays are recommended to monitor shifts of the innervation zone and select proper channels for analysis of long muscles. Variable load sharing among muscles is likely present.
Selection of epoch duration	1	Epoch duration and reasons for selecting it	At low contraction levels the sEMG signal may be non-stationary because of MU rotation and at high contraction level is non-stationary because of MMMF (section 7).
Method for spectral estimation	1	Spectral estimation modality (e.g., single epoch periodogram, multiple epoch Welch periodogram and degree of epoch overlapping) and frequency resolution.Amount of zero-padding, if used to extend the epoch duration.	The PSD of a sEMG single epoch is very jagged. Smoothing may be applied by taking sub-epochs and then averaging the spectra obtained from them, at the cost of poorer frequency resolution. Alternatively, the jagged spectrum may be smoothed by filtering the spectrum itself (e.g., by moving average).

information from frequency analysis of the voluntary sEMG are largely overlapping with those required for amplitude analysis, that can be found in (Clancy et al., 2023; Vowels, Vowels and Wood, 2023; Besomi et al., 2024) and a number of elementary (Barbero, Merletti and Rainoldi, 2012; Butz, 2015) or intermediate textbooks (Samani, 2019).

There are many ways to estimate the sEMG amplitude or PSD. The choice of the modalities to use depends on the purpose of the test or clinical evaluation to be performed (e.g., isometric constant force or variable force contractions, fast or slow dynamic contractions, short or long muscles, etc). In most applications frequency analysis is performed to monitor and quantify MMMF or to quantify EEG-EMG or EMG-EMG coherence.

It should be underlined that, when the sEMG features of different subjects are compared, applying the same force/torque (e.g., holding the same weight in the hand to study the biceps brachii) implies a different % MVC for each subject (or arm) and MMMF and time to failure may be different for this reason. Isometric braces with torque meters or load cells should be used, MVC should be measured (Colombo et al., 2000; Oddsson and De Luca, 2003) and targets at the desired percentage of MVC should be set on a screen and matched by the subject.

The user must be familiar with concepts illustrated in previous sections of this and of other tutorials and summarized below:

1. Raw sEMG is a 2D signal (a map) evolving in time. Each monopolar or single differential (bipolar) raw sEMG is a random signal (channel) which is quasi-stationary or non-stationary and is a sample of the map, in a specific spatial location, evolving in time.
2. Frequency analysis of sEMG is usually performed as a bench-test, similar to an ECG stress test. Performing it during a dynamic activity is very critical and requires great care and more advanced analysis, for instance based on time–frequency distributions (not discussed in this tutorial).
3. Amplitude and frequency features of a sEMG channel are evolving in time and highly dependent from electrode geometry and anatomical factors (Fig. 10 and (Merletti and Muceli, 2019; Clancy et al., 2023)).
4. Amplitude and frequency features computed over selected time epochs of a sEMG channel are random variables themselves and are never the same from epoch to epoch even for stationary signals. They

are estimates which must be fitted by regression lines or curves to be able to observe and quantify changes and trends. Trends, rather than individual epoch values, are of interest.

5. Epoch to epoch fluctuations of frequency features depend on the contraction level and the epoch duration. If the sEMG signal is quasi-stationary over a few epochs, partial overlapping of subsequent epoch reduces the variance of the estimates. If the epochs must be short because of rapidly varying force or dynamic contractions, artificial epoch lengthening by zero padding reduces the variability of the frequency estimates but does not increase the information and is almost a purely "cosmetic" improvement.

Table 1 provides a checklist for preparing/reviewing reports and manuscripts dealing with sEMG frequency analysis.

9. Concluding remarks

This tutorial focuses on fundamental concepts concerning sEMG frequency analysis and is complemented by other tutorials on signal detection and conditioning, amplitude and MFCV estimation. The fundamental articles on frequency analysis of sEMG go back more than 50 years. Nevertheless, clinical applications are lacking possibly because of the lack of knowledge of the information that sEMG can provide and the lack of standardization required by this technique. Protocols for testing procedures, modalities of spectral estimation and interpretation of the results lack standardization which needs to be developed by engineers and clinicians on the basis of the experience acquired in clinical research institutions.

Frequency analysis of sEMG must be integrated with amplitude analysis (Clancy et al., 2023) and, because of load sharing, should concern all the muscles acting on a joint (agonists and antagonists). This is not yet possible today but, at least for superficial muscles, this is often achieved using electrode grids covering the muscles of interest. Despite recent progresses some information cannot be obtained from the sEMG PSD although in particular cases or test conditions it can be surmised. Some examples are listed below:

1. MU firing rates and recruitment/de-recruitment strategies
2. Number of active MUs and their location (depth) or their histological type or territory
3. Force produced by individual muscles

However, other techniques (e.g., decomposition of sEMG into the constituent MUAP trains (Holobar and Zazula, 2007; Chen and Zhou, 2016; Negro et al., 2016)) are needed for investigating recruitment/de-recruitment and rate coding.

Reproducibility of measurements is at the heart of science. Reporting complete information to allow reproduction of a test by others is a fundamental good practice.

CRediT authorship contribution statement

Silvia Muceli: Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Roberto Merletti:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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