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Frequency-Dependent Uncertainty Analysis of Bioimpedance in Engineered and Native Muscle

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ABSTRACT Bioimpedance-based characterization of engineered muscle systems is inherently affected by frequency-dependent uncertainty, which limits measurement reliability and interpretability in biomedical and bioinspired applications. A systematic understanding of uncertainty propagation across frequency can therefore benefit the robustness of measurements and enable more consistent comparisons between biological tissues, where an unavoidable source of variability arises from the intrinsic biological nature of the specimens. In this work, a frequency-resolved uncertainty framework was applied to impedance measurement of an engineered muscle system (X-MET) and a native muscle reference, both obtained from a murine model. The method integrated instrumental, repeatability, and biological variability contributions to provide a structured decomposition of total uncertainty in the frequency domain, enabling consistent evaluation of reliability metrics such as relative uncertainty. Importantly, uncertainty analysis was extended beyond electrical measurements to include tissue imaging data, since sample dimensional variability was explicitly quantified and incorporated into the normalized impedance evaluation. This allowed the effect of geometric variability to be consistently propagated within the uncertainty model. Results demonstrated that both engineered and native systems exhibit frequency-dependent behavior, with increasing relative uncertainty at higher frequencies and nonuniform contributions across uncertainty sources. Distinct frequency regimes were identified, characterized by different levels of measurement reliability and signal quality. The engineered system showed reproducible and well-defined uncertainty patterns across the spectrum, despite exhibiting higher relative uncertainty than native muscle. Nevertheless, consistent frequency-dependent trends were preserved, supporting the identification of operational frequency regions despite intrinsic biological variability.

INDEX TERMS Bioimpedance spectroscopy, engineered muscle, frequency-dependent systems, measurement uncertainty analysis.

I. INTRODUCTION

SKELETAL muscle is the largest organ in the human body and plays a central role not only in movement but also in whole-body metabolic homeostasis [1], [2]. It is a highly structured and anisotropic tissue, composed of aligned multinucleated fibers embedded in an extracellular matrix and organized across multiple spatial scales. This hierarchical organization, together with the coexistence of metabolically heterogeneous fiber types, makes skeletal muscle particularly

difficult to reproduce in vitro [1], [3]. In this context, tissue engineering (TE) has emerged as a promising strategy to generate 3-D constructs that mimic native muscle. However, reproducing its complex architecture and contractile function remains a major challenge [4], [5], [6]. Therefore, reliable quantitative methods for monitoring engineered muscle during maturation are essential, particularly for translational applications. Conventional characterization techniques, such as histological and molecular analyses, provide detailed

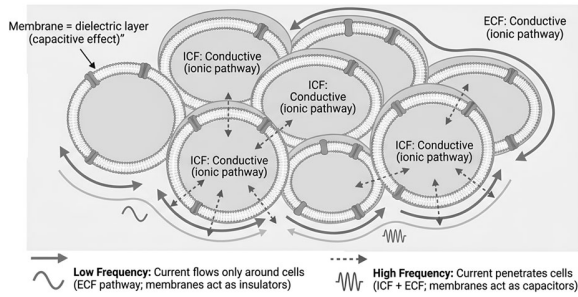


FIGURE 1. Equivalent electrical representation of biological tissue showing conductive fluids and capacitive cell membranes.

information on cellular organization but are destructive, time-consuming, and unsuitable for repeated measurements [7], [8]. Consequently, nondestructive, label-free approaches capable of quantitatively monitoring tissue structure and function are gaining increasing interest [9], [10], [11]. Among these, bioelectrical impedance analysis is a powerful nondestructive tool because tissue electrical response arises from the combined properties of intracellular fluids (ICFs), extracellular fluids (ECFs), and cell membranes [12]. ICF and ECF provide conductive ionic pathways, whereas cell membranes, composed of lipid bilayers with embedded proteins, behave as dielectric layers, introducing capacitive effects. Consequently, biological tissues exhibit a complex impedance resulting from the resistive ionic conduction and membrane polarization [12], [13], [14], [15], as schematically shown in Fig. 1. Importantly, the electrical response depends on the applied alternating current and frequency range. At low frequencies, current flow is mainly confined to extracellular pathways due to membrane insulation, whereas at higher frequencies it progressively penetrates intracellular regions. This frequency-dependent behavior enables the investigation of different structural and functional features [12], [13], [16], [17], [18], [19]. Moreover, impedance varies among tissues and within the same tissue, according to composition, fiber orientation, membrane integrity, extracellular matrix, and physio/pathological conditions. In anisotropic tissues such as skeletal muscle, electrical properties are further influenced by fiber orientation, leading to direction-dependent responses [2], [15]. This relationship between electrical properties and tissue structure makes impedance analysis a valuable tool for tissue assessment. These features are particularly advantageous for engineered skeletal muscle, whose structure and function evolve over time. Impedance-based techniques enable rapid, repeatable, and nondestructive monitoring of the same construct. Impedance magnitude ($|Z|$) reflects resistive and capacitive contributions of intra- and extra-cellular pathways, the phase angle (θ) describes their balance and is associated with cellular integrity, whereas the dissipation factor (D) quantifies dielectric losses related to membrane and fluid properties [20], [21], [22], [23]. In our previous work, these parameters were exploited to

characterize, for the first time, the dielectric behavior of an ex vivo engineered skeletal muscle construct (X-MET) [24], [25] through multifrequency electrical impedance measurements. Bioimpedance parameters acquired from X-MET were compared with those of native murine soleus muscle (SOL) to provide a quantitative assessment of the engineered tissue electrical functionality. The soleus was selected as the closest physiological counterpart in terms of histological and mechanical properties, based on previous functional analyses [24], [26], [27], [28]. Beyond serving as a physiological reference, SOL provides a metrological benchmark to distinguish biological variability from measurement-related uncertainty, assessing the electrical reliability of the engineered construct.

This article extends the conference paper by Ingrosso et al. [29]. While the previous work focused on the dielectric characterization of X-MET through multifrequency impedance measurements, the present work addresses experimental uncertainty quantification, a critical yet often overlooked aspect of bioimpedance measurements. The main advancement with respect to the previous conference contribution is the transition from impedance characterization to a metrological evaluation of measurement reliability. While the previous study established the dielectric response of X-MET, the present work quantifies the uncertainty associated with impedance measurements and identifies the frequency ranges where engineered muscle measurements provide statistically and metrologically reliable information. Although uncertainty analysis is well established in impedance spectroscopy, previous studies have mainly focused on instrumentation performance, calibration procedures, equivalent-circuit parameter estimation, and uncertainty propagation in electrochemical systems [30], [31], [32]. In bioimpedance, research has primarily addressed tissue electrical behavior, electrode polarization, and biological variability, whereas uncertainty has rarely been used as a metrological criterion to assess the reliability of biological tissue measurements [33]. Similarly, although impedance-based characterization of engineered skeletal muscle has been demonstrated [29], [34], [35], a frequency-resolved uncertainty analysis identifying reliable measurement conditions is still lacking.

Specifically, this work proposes a frequency-resolved metrological framework for engineered skeletal muscle integrating instrumental accuracy, repeatability, biological reproducibility, and geometrical uncertainty. Rather than simply quantifying measurement error, the framework identifies the frequency ranges in which bioimpedance measurements remain metrologically reliable and compares the uncertainty structure of engineered and native muscle, providing a stronger quantitative basis for bioimpedance-based tissue characterization.

II. MATERIALS AND METHODS

A. TISSUE IMPEDANCE MEASUREMENTS EXPERIMENTAL SETUP

Experimental data used for the uncertainty analysis were collected from previous impedance measurement tests

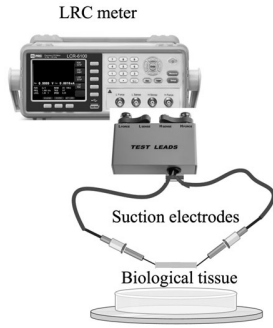


FIGURE 2. Schematic of the system employed for tissue bioimpedance testing.

performed on wild-type (C57BL-6) SOLs and X-MET samples [29]. Briefly, X-METs are scaffold-free engineered skeletal muscle tissues generated from primary cultures of murine hindlimb muscle cells [24], [25]. They reproduce key structural and functional features of native skeletal muscle, including aligned fibers, spontaneous contractions, and physiological responses to electrical stimulation, making them suitable for electromechanical characterization [24], [25]. The experimental setup for electrical impedance measurements was specifically developed and is schematically illustrated in Fig. 2. The system was based on an RLC meter (RLC-6100, GW Instek) operating in a four-wire (tetrapolar) configuration. RS PRO LCR Meter Chip Test Fixture cables connected the instrument to two suction electrodes (A-M Systems Inc., Sequim, WA), which gently secured the tissue ends through stable and nondamaging mechanical adhesion. The tetrapolar configuration allowed spatial separation between current injection and voltage sensing, thereby substantially reducing the influence of electrode–tissue interface impedance and electrode polarization, particularly at low frequencies, compared with conventional two-electrode measurements. A microscope monitored tissue positioning and suction stability throughout the measurements. Tissue geometry was recorded using a PC-based acquisition system equipped with a frame grabber, LabVIEW software, and a factory-calibrated Basler acA2040-180 km camera coupled with a Nikon SMZ800 stereomicroscope, for dimensional assessment and impedance normalization. The experimental dataset comprised five X-METs and four SOL specimens, all tested on the same day. For dielectric characterization, tissues were positioned in PBS and secured by the suction electrodes. The PBS was then removed, and impedance measurements were performed in air to minimize the contribution of the surrounding conductive medium, allowing the measured impedance to predominantly reflect tissue electrical properties [36], [37].

Impedance measurements were carried out under low-amplitude excitation conditions to avoid excessive stimulation and tissue damage. The RLC meter was operated in $|Z|$ – D mode, from which θ was also derived. Measurements were performed over a frequency range from 10 Hz to 80 kHz

in 10 Hz increments, a range commonly used for biological tissues [20], [38]. For each frequency point, three consecutive measurements were acquired. The number of repetitions was intentionally limited to minimize time-dependent changes (e.g., hydration and viability), so that the observed results primarily reflected short-term measurement precision under nominally unchanged conditions [39], [40], [41]. Data were analyzed in MATLAB. Outliers were identified using a 3σ criterion and replaced with NaN values [42], [43]. The robustness of the outlier identification was additionally verified using the median absolute deviation (MAD) criterion, which confirmed the same anomalous values [44], [45]. Mean values, standard deviations, and root mean square (rms) uncertainties were computed using NaN-aware functions.

B. TISSUE IMPEDANCE MEASUREMENTS UNCERTAINTY

To enhance the reliability of the impedance measurements, the main sources of uncertainty associated with the measurement system, experimental setup, repeatability, and reproducibility were considered. The core of the setup was the RLC meter, whose instrumental uncertainty was evaluated from the certified manufacturer specifications for the GW Instek RLC-6100 series [46], considering both impedance magnitude and dissipation factor (D) accuracies, and treated as a Type B contribution, in agreement with previous impedance and S-parameter studies [32], [47], [48], [49]. Type A uncertainty contributions were evaluated experimentally: repeatability under unchanged experimental conditions [32], [47], and reproducibility from different biological specimens, accounting for intersample variability [40], [47], [50]. Owing to the biological nature of the specimens, the latter reflects intrinsic biological heterogeneity. Following the approach presented in the GUM, the coverage factor was determined from the Student's t -distribution according to the effective degrees of freedom (ν_{eff}). For each test condition, ν_{eff} was evaluated considering $\nu_{X-MET} = 4$ and $\nu_{SOL} = 3$, while the instrumental contribution was assigned infinite degrees of freedom [51]. The coverage factor was computed as $k = t_{0,\nu_{eff}}$ corresponding to a 68.27% confidence level, and the expanded uncertainty was obtained as $u_E = k \cdot u$, where u refers to the repeatability and reproducibility uncertainty contribution, which are hereafter denoted as $u_{rep}^{rms}(f)$ and $u_{bio}(f)$, respectively [52], [53]. $K = 1.20$ for X-MET and 1.14 for SOL. Assuming independent contributions, the combined standard uncertainty was calculated using the rms law of propagation of uncertainty [32], [47], [54]. The uncertainty associated with the normalized $|Z|$ values also included the contribution related to specimen's geometry [55], [56], [57], [58]. The individual uncertainty components are described in Sections II-B1–II-B4.

1) INSTRUMENTAL UNCERTAINTY

Instrumental uncertainty was quantified from the impedance magnitude accuracy specifications reported for the RLC analyzer [46]. The corresponding accuracy model for $|Z|$

TABLE 1. Instrumental accuracy model parameters.

Symbol	Description
A	Basic measurement accuracy
A_r	Basic accuracy correction factor (amplitude-dependent)
K_a	Impedance factor a
K_b	Impedance factor b
K_f	Open/short trimming factor
K_c	Temperature correction factor
K_L	Test lead length factor

measurements is given by the following equation:

$$A_e = \pm [A \cdot A_r + (K_a + K_b + K_f) \cdot 100 + K_L] \cdot K_C [\%] \quad (1)$$

where A_e represents the relative accuracy of the measured impedance magnitude. The meaning of each parameter is summarized in Table 1. The basic accuracy A depends on the excitation signal amplitude V_s and the selected measurement speed. During measurements, $V_s = 0.5V_{rms}$, and the instrument operated in slow/medium mode, yielding $A = 0.05$. Since the applied excitation amplitude falls within the nominal operating range ($0.4V_{rms} \leq V_s \leq 1.2V_{rms}$), $A_r = 1$. Since $|Z| > 500 \Omega$, K_a was neglected and only K_b was considered [46]. K_b depends on frequency and measured impedance, with separate expressions for the low-frequency region ($f < 100$ Hz) and high-frequency region ($f \geq 100$ Hz), respectively, according to the following equations:

$$K_b(Z, f) = Z_m \cdot 10^{-9} \cdot \left(1 + \frac{70}{V_s}\right) \cdot \left(1 + \sqrt{\frac{100}{f}}\right) \quad (2)$$

$$K_b(Z, f) = Z_m \cdot 10^{-9} \cdot \left(1 + \frac{70}{V_s}\right) \quad (3)$$

where f is the excitation frequency, and Z_m is the measured impedance. This formulation ensures that the instrumental uncertainty was consistently propagated across the entire frequency spectrum of interest. The remaining correction factors were set to $K_C = 1$, corresponding to controlled laboratory conditions (18 °C–28 °C); $K_f = 0$, due to calibration at the measurement frequencies; $K_L = 0$, owing to short leads (<1 m). Hence, (1) reduced to the following equation:

$$A_e(f) = \pm [A \cdot A_r + K_b(f) \cdot 100] [\%]. \quad (4)$$

The relative uncertainty $A_e(f)$ was evaluated at each frequency point. All terms of (1) and (4) are dimensionless, ensuring that A_e is expressed as a percentage. Although K_b depends on Z_m , this dependence does not introduce physical units but acts as an internal scaling variable defining the applicable operating region of the correction model. Hence, K_b must be interpreted as a dimensionless correction factor, fully consistent with the dimensional structure of (1). The

same approach was applied to the dissipation factor D , yielding the following equation [46]:

$$D_e = f(x) = \begin{cases} \pm A_e, & D \leq 0.1 \\ \pm A_e (1 + D), & D > 0.1. \end{cases} \quad (5)$$

After converting the relative accuracy from percentage to fractional form, a rectangular probability distribution was assumed. The instrumental standard uncertainty of $|Z|$ was then calculated according to the following equation:

$$u_{ins,|Z|}(f) = \frac{A_e}{\sqrt{3}} \cdot Z_m(f) \quad (6)$$

where $Z_m(f)$ is the measured impedance magnitude at each frequency point. The instrumental uncertainty of D was obtained analogously, as reported in the following equation:

$$u_{ins,D}(f) = \frac{D_e}{\sqrt{3}} \cdot D_m(f) \quad (7)$$

where $D_m(f)$ is the measured dissipation factor. The same procedure was applied to both X-MET and SOL datasets.

2) REPEATABILITY UNCERTAINTY

Repeatability was evaluated to quantify the short-term variability of the impedance measurements under unchanged conditions on the same tissue sample. This Type A contribution accounts for random fluctuations arising from the measurement system, electrode–tissue interface, and environmental noise. For each biological sample and frequency point, $n = 3$ repeated measurements were performed. The repeatability standard deviation was computed according to the following equation:

$$s_j(f) = \sqrt{\frac{1}{n-1} \sum_{k=1}^n (x_{j,k}(f) - \bar{x}_j(f))^2} \quad (8)$$

where $x_{j,k}(f)$ is the k th repeated measurement of sample j , $\bar{x}_j(f)$ is the corresponding sample mean, and n is the number of repetitions. Since the measurand was estimated as the mean of three repetitions, the corresponding standard uncertainty contribution was calculated as: $u_{rep,j}(f) = s_j(f)/\sqrt{3}$, yielding the repeatability uncertainty for each specimen and frequency point. The overall repeatability contribution at each frequency was obtained by rms aggregation across the biological specimens:

$$u_{rep}^{rms}(f) = \sqrt{\frac{1}{N} \sum_{j=1}^N u_{rep,j}^2(f)} \quad (9)$$

where N is the number of biological specimens. Repeatability was first evaluated for each specimen and then aggregated across biological samples using the rms approach, yielding a frequency-dependent estimate of the average intra-sample measurement stability [40], [45]. The resulting contribution was propagated into the combined and expanded uncertainty ($u_{E,rep}^{rms}(f)$) according to the procedure described above. The same approach was applied to both $|Z|$ and D , consistent with standard practices in impedance spectroscopy and bioimpedance measurements [54], [59].

3) REPRODUCIBILITY UNCERTAINTY

Reproducibility was evaluated to quantify the variability across different biological specimens. For each frequency point, the corresponding standard uncertainty was calculated as the standard deviation of the specimen means, divided by the square root of the number of specimens ($N = 5$ for X-MET and $N = 4$ for SOL) as shown in the following equation:

$$u_{bio}(f) = \frac{std(\bar{x}(f))}{\sqrt{N}}. \quad (10)$$

The expanded uncertainty was evaluated following the procedure described above ($u_{E,bio}(f)$). The same approach was applied to both $|Z|$ and D . This contribution reflects biological heterogeneity and the uncertainty associated with estimating the population means from a finite number of specimens, rather than instrumental error. In biological tissues, this term is intrinsically significant due to unavoidable physiological heterogeneity and structural variability among samples. Assuming mutual statistical independence, (11) gives the combined standard uncertainty for both $|Z|$ and D , obtained by quadratic propagation of the uncertainty contributions defined in Sections II-B1–II-B3

$$u_{parameter}(f) = \sqrt{(u_{ins}(f))^2 + (u_{E,rep}^{rms}(f))^2 + (u_{E,bio}(f))^2} \quad (11)$$

where *parameter* denotes either $|Z|$ or D .

4) GEOMETRICAL UNCERTAINTY

Impedance values were normalized to account for tissue geometry, introducing additional uncertainty associated with the geometrical parameters. Normalization was performed as $Z_{norm} = Z \cdot S/L$, where S and L are, respectively, the tissue cross-sectional area and longitudinal length. Following [53], uncertainty in L (u_L) and S (u_S) were evaluated from optical measurements, treated as frequency-independent quantities and propagated to the normalized impedance (12). Accordingly, covariance terms were neglected, as no statistical dependence between the geometrical and electrical quantities was assumed, consistent with the GUM framework:

$$u_{norm} = \sqrt{\left(\frac{u_Z(f)}{Z(f)}\right)^2 + \left(\frac{u_S}{S}\right)^2 + \left(\frac{u_L}{L}\right)^2} \quad (12)$$

where $u_Z(f)$ is the combined impedance uncertainty.

Geometrical quantities (tissue length and diameter) were measured in pixels using ImageJ and converted into physical dimensions using a calibrated pixel-to-length conversion based on a reference grid. Based on our prior experience [60], pixel detection uncertainties of ± 2 pixels for each endpoint of the reference grid and ± 5 pixels for each endpoint of the specimen length were assumed, as shown in Fig. 3(a). Assuming rectangular distributions, the corresponding standard uncertainties were obtained by dividing these values by $\sqrt{3}$, yielding $u_{grid} = 1.63$ pixels and $u_{pix,L} = 4.08$ pixels. The specimen diameter was estimated as the average of

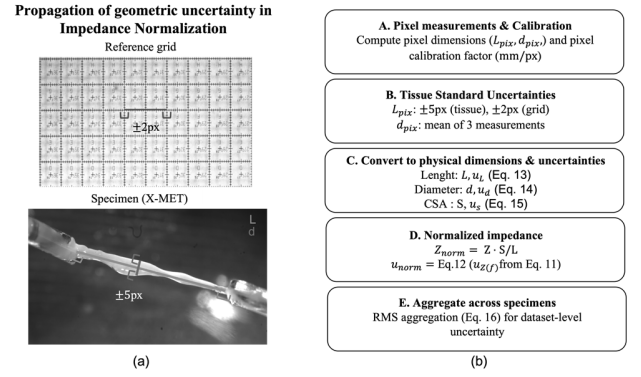


FIGURE 3. (a) Pixel-based measurement procedure showing the reference grid used for calibration and the tissue specimen used for geometrical parameter extraction. Endpoint detection uncertainties are indicated for both measurements. (b) Workflow for uncertainty propagation from pixel measurements to normalized impedance.

three measurements acquired at 25%, 50%, and 75% of the specimen length. Assuming a standard uncertainty of 4.08 pixels for each measurement, propagation of the three diameter uncertainties yielded $u_{pix,d} = 2.36$ pixels. Following the GUM framework, and assuming rectangular distributions, these uncertainties were propagated to the physical dimensional quantities according to the following equations:

$$u_L = L \sqrt{\left(\frac{u_{pix,L}}{L_{pix}}\right)^2 + \left(\frac{u_{grid}}{L_{grid}}\right)^2} \quad (13)$$

$$u_d = d \sqrt{\left(\frac{u_{pix,d}}{d_{pix}}\right)^2 + \left(\frac{u_{grid}}{L_{grid}}\right)^2}. \quad (14)$$

The mean diameter was subsequently used to calculate the cross-sectional area assuming a cylindrical geometry, from which the associated uncertainty u_S was obtained by propagating the following equation:

$$u_S = \frac{\pi}{2} \cdot d \cdot u_d. \quad (15)$$

For each specimen, geometrical uncertainty (u_{geom}) was first evaluated using (12) and then aggregated across specimens by rms using the following equation:

$$u_{geom} = \sqrt{\frac{1}{N} \sum_{i=1}^N u_{norm}^2}. \quad (16)$$

This rms-based approach was preferred over a worst case estimate, as it provides a more realistic representation of the overall geometrical uncertainty across specimens.

The optical measurement procedure and the subsequent uncertainty propagation steps are illustrated in Fig. 3(b). The reference grid and tissue specimen images provide the basis for pixel calibration and geometrical parameter extraction, respectively.

III. RESULTS

A. IMPEDANCE MAGNITUDE

Fig. 4 reports the frequency-dependent impedance magnitude for X-MET and SOL, together with their associated

TABLE 2. Variance-based relative contribution of uncertainty sources for impedance magnitude (Z).

F (Hz)	Instrumentation				Repeatability				Reproducibility				Combined uncertainty	
	X-MET		SOL		X-MET		SOL		X-MET		SOL		X-MET	SOL
	Ω	%	Ω	%	Ω	%	Ω	%	Ω	%	Ω	%	Ω	Ω
10	1.52 · 10 ³	1.65	76.6	0.38	4.59 · 10 ³	15.05	41.0	0.11	1.08 · 10 ⁴	83.30	1.23 · 10 ³	99.50	1.18 · 10 ⁴	1.24 · 10 ³
100	3.17 · 10 ²	0.088	17.6	0.026	2.26 · 10 ³	4.49	42.3	0.15	1.04 · 10 ⁴	95.42	1.09 · 10 ³	99.82	1.07 · 10 ⁴	1.09 · 10 ³
1000	3.23 · 10 ²	0.069	14.5	0.021	6.51 · 10 ²	0.28	16.9	0.029	1.23 · 10 ⁴	99.65	9.92 · 10 ²	99.95	1.23 · 10 ⁴	9.92 · 10 ²
10000	3.08 · 10 ²	0.056	12.8	0.022	8.43 · 10 ²	0.42	10.1	0.014	1.30 · 10 ⁴	99.52	8.69 · 10 ²	99.96	1.30 · 10 ⁴	8.69 · 10 ²
50000	1.66 · 10 ²	0.036	10.2	0.027	2.97 · 10 ²	0.12	11.7	0.035	8.68 · 10 ³	99.85	6.23 · 10 ²	99.94	8.70 · 10 ³	6.24 · 10 ²

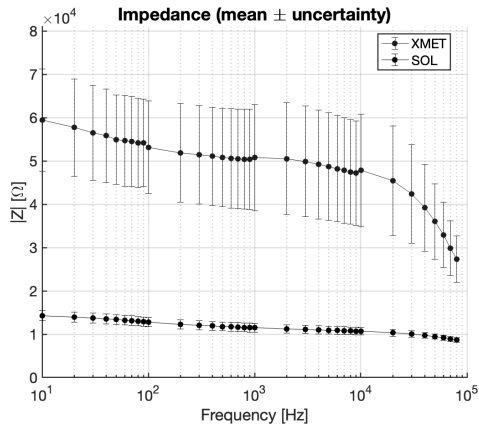


FIGURE 4. Impedance magnitude and expanded uncertainty for X-MET and SOL.

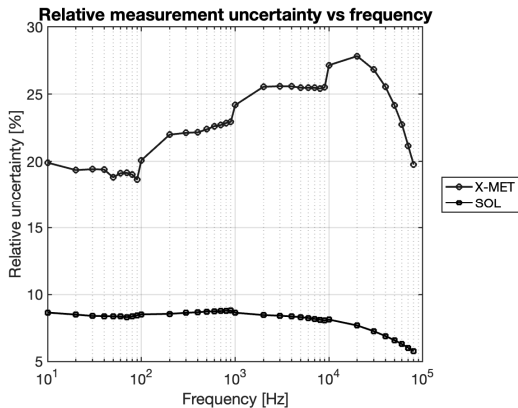


FIGURE 5. Relative uncertainty for X-MET and SOL as a function of frequency.

expanded uncertainties obtained through (11). In both systems, the impedance magnitude decreases with frequency over the investigated range. X-MET exhibits average values decreasing from approximately $5.95 \times 10^4 \Omega$ at 10 Hz to $2.73 \times 10^4 \Omega$ at 80 kHz, while SOL shows lower impedance values, decreasing from approximately $1.43 \times 10^4 \Omega$ to $8.67 \times 10^3 \Omega$. In addition, the native muscle exhibits lower absolute uncertainty than X-MET across the full frequency band. In both cases, uncertainty contributions are more pronounced at low frequencies and progressively decrease toward higher frequencies. Table 2 shows the relative variance contributions of instrumental effects, repeatability, and

biological variability. This representation allowed a more detailed assessment of the uncertainty structure, independently of the absolute impedance values, providing a clearer view of variability-dominant sources across the investigated frequency range. Results show that the relative contributions of the different uncertainty sources vary with frequency and between samples. For X-MET, at 10 Hz the uncertainty is dominated by reproducibility (83.30%), followed by repeatability and instrumentation, whereas at 50 kHz reproducibility accounts for 99.85% of the total uncertainty. A similar trend is observed for SOL, where reproducibility is the dominant contribution across all frequencies, ranging from 99.50% at 10 Hz to 99.94% at 50 kHz. Instrumentation and repeatability contributions remain consistently below 7% over most of the frequency range. To further evaluate measurement reliability independently of the absolute impedance magnitude, the relative uncertainty was analyzed as a function of frequency, as reported in Fig. 5. SOL exhibits values in the range of approximately 5.75%–8.75%, with a slight decreasing trend at higher frequencies, whereas X-MET shows higher values, approximately between 21% and 31%, with a more pronounced variation over the investigated frequency range.

The relative uncertainty profiles show small discontinuities at frequency-decade transitions. These features are mainly attributable to the acquisition procedure, as measurements were performed in successive frequency blocks rather than in a single continuous sweep, requiring separate instrument settings for different frequency decades. Consequently, slight shifts in the estimated uncertainty may occur at decade boundaries. However, these slight discontinuities do not affect the main uncertainty effects and relationship among the two tissues. In addition, the larger fluctuations observed in X-MET are likely related to the intrinsic structural heterogeneity of the engineered construct. Compared with native muscle tissue, which exhibits a smoother frequency response, X-MET is more sensitive to experimental variability, resulting in greater changes in relative uncertainty across the investigated frequency range.

B. DISSIPATION FACTOR

Fig. 6 show shown mean $\pm$ rms uncertainty of D computed for X-MET and SOL muscle. X-MET shows average D values ranging from approximately 8.6–24.3 in the low-to-mid frequency range, followed by a pronounced

TABLE 3. Variance-based relative contribution of uncertainty sources for dissipation factor (D).

F (Hz)	Instrumentation				Repeatability				Reproducibility				Combined uncertainty	
	X-MET		SOL		X-MET		SOL		X-MET		SOL		X-MET	SOL
10	1.78	91.74%	0.42	35.83%	0.42	5.18%	0.25	12.58%	0.33	3.08%	0.50	51.58%	1.86	0.70
100	1.62	37.39%	0.082	11.39%	0.85	10.27%	0.12	24.57%	1.91	52.34%	0.19	64.03%	2.64	0.24
1000	2.97	82.19%	0.27	14.10%	0.25	0.57%	0.037	0.25%	1.36	17.24%	0.68	85.65%	3.28	0.73
10000	1.42	25.45%	0.53	1.28%	0.33	1.34%	0.055	0.014%	2.41	73.21%	4.67	98.71%	2.82	4.70
50000	8.54	29.02%	0.51	0.99%	2.06	1.69%	0.10	0.038%	13.20	69.29%	5.15	98.96%	15.86	5.18

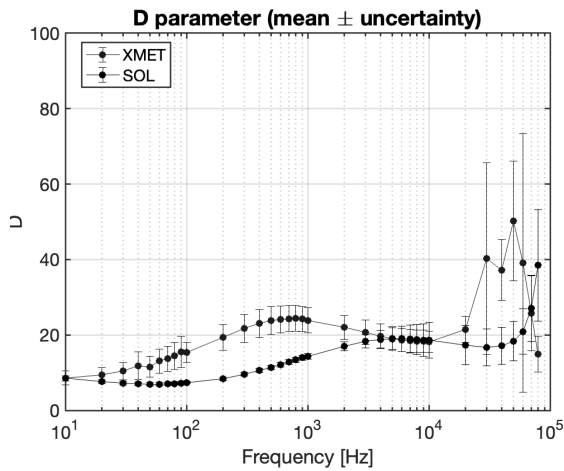


FIGURE 6. Mean and rms uncertainty of parameter D for X-MET and SOL as a function of frequency.

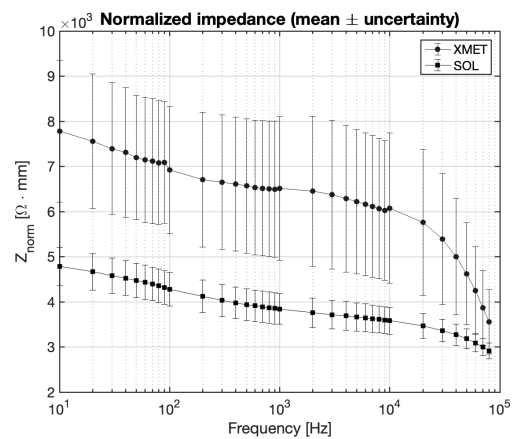


FIGURE 8. Normalized impedance magnitude and expanded uncertainty for X-MET and SOL as a function of frequency.

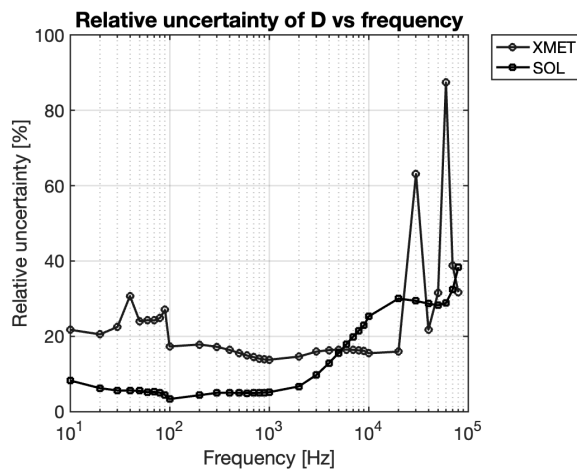


FIGURE 7. Relative uncertainty of parameter D for X-MET and SOL as a function of frequency.

increase between 30 and 60 kHz, reaching a maximum at 50 kHz.

The SOL muscle exhibits lower average values at low frequencies (approximately 6.9–8.5), followed by a gradual increase with frequency up to approximately 38.5 at the highest frequency. Regarding uncertainty, X-MET exhibits larger uncertainty bars across the entire frequency range compared to muscle. While the uncertainty remains

relatively bounded at low and mid frequencies, a pronounced increase is observed in the high-frequency range (30–60 kHz). SOL, on the other hand, shows smaller uncertainty values at low frequencies, followed by a progressive increase toward higher frequencies. Table 3 reports the variance-based decomposition of uncertainty contributions for the dissipation factor D, while Fig. 7 shows the relative uncertainty as a function of frequency. X-MET exhibits relatively stable values ($\approx 15\%$ – 24%) over the low-to-mid frequency range, followed by a strong increase in the high-frequency region, with pronounced peaks at 30 and 60 kHz reaching up to $\sim 63\%$ and $\sim 87\%$, respectively. SOL exhibits low relative uncertainty at low frequencies ($<10\%$), reaching a minimum around 100 Hz, followed by a progressive increase with frequency up to approximately 38% at the highest frequencies.

C. NORMALIZED IMPEDANCE MAGNITUDE

Fig. 8 reports the frequency-dependent normalized impedance magnitude for both X-MET and SOL, together with their associated expanded uncertainties. Compared with the absolute impedance representation, normalization reduces the difference between the two datasets. In the normalized representation, X-MET decreases from approximately $7.8 \times 10^3 \Omega \cdot \text{mm}$ at 10 Hz to $3.6 \times 10^3 \Omega \cdot \text{mm}$ at 80 kHz, whereas SOL decreases from approximately $4.8 \times 10^3 \Omega \cdot \text{mm}$ to $2.9 \times 10^3 \Omega \cdot \text{mm}$ over the same frequency range.

Therefore, although X-MET remains systematically higher than SOL across the entire frequency range investigated, the separation between the two datasets is substantially reduced after geometrical normalization. Moreover, the associated uncertainty intervals are generally closer than in the absolute representation and overlap only at the lowest frequencies, indicating a reduced distinction between the two normalized impedance measurements. Fig. 9 reports the relative uncertainty associated with the normalized impedance measurements. For X-MET, the relative uncertainty ranges approximately between 19% and 28%. Values remain close to 20% at low frequencies, increase progressively up to a maximum of approximately 28% in the kHz range, and then decrease toward approximately 20% at the highest frequencies. For SOL, the relative uncertainty remains close to 9% at low frequencies and progressively decreases with frequency, reaching approximately 6% at the highest frequency. After normalization, both systems maintain similar qualitative trends, and relative uncertainty remains comparable to that observed for the absolute impedance, indicating that the normalization procedure does not substantially increase the overall measurement uncertainty. SOL exhibits lower and more homogeneous uncertainty levels, with smooth frequency-dependent variations and limited localized fluctuations. In contrast, X-MET shows systematically higher uncertainty magnitudes, with more pronounced frequency-dependent variability and isolated peaks, particularly in the high-frequency range.

Despite these differences in absolute magnitude and distribution, both systems preserve well-defined and reproducible frequency trends in the measured parameters, indicating that the observed behavior is not dominated by random fluctuations but reflects stable underlying electrical responses. In this context, uncertainty primarily affects the precision of the estimates rather than the overall shape of the frequency-dependent profiles.

IV. DISCUSSION

The uncertainty analysis performed in this study provides important insight into the reliability and robustness of the electrical characterization of both native muscle and the engineered construct. Although X-MET exhibits higher uncertainty levels compared to SOL, the overall results demonstrate that the engineered tissue can reproduce not only the expected impedance behavior, but also a coherent and physically meaningful uncertainty structure across frequency. In both systems, impedance magnitude decreases with frequency, confirming that X-MET successfully captures the characteristic dispersive response typically observed in biological muscle tissue. Importantly, the engineered construct maintains this behavior consistently over the investigated bandwidth despite its more complex and less physiologically consolidated structure. This indicates that the observed electrical response is not random or measurement-driven but reflects stable underlying tissue properties. The higher

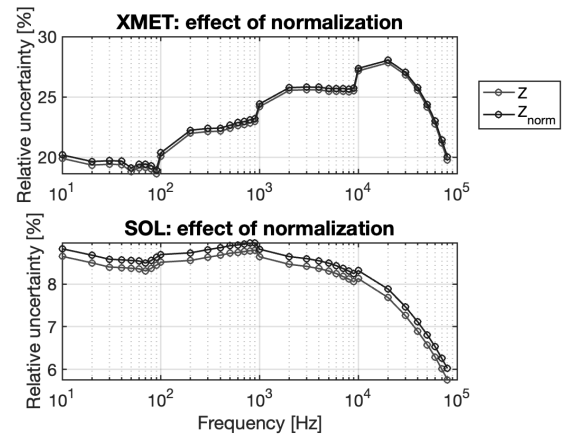


FIGURE 9. Relative uncertainty of normalized Z for X-MET and SOL.

uncertainty associated with X-MET should therefore be interpreted in the context of the intrinsic nature of engineered biological systems. As an ex vivo reconstructed tissue, X-MET is expected to exhibit greater sample-to-sample variability than native muscle, due to differences in cellular organization, extracellular matrix development, and microstructural maturation during tissue formation. Unlike native muscle, which benefits from highly organized and physiologically optimized architecture, engineered tissues are inherently characterized by greater structural variability and evolving microstructural organization. According to our study, the uncertainty levels observed in X-MET remain controlled and sufficiently bounded to preserve clear frequency-dependent trends and acceptable signal-to-uncertainty ratios over most of the spectrum. Comparison with native muscle therefore provides more than a reference dataset, allowing the electrical reliability and uncertainty of the engineered construct to be interpreted within the context of physiological tissue variability. The variance decomposition further supports this interpretation. For impedance magnitude, both X-MET and SOL are predominantly characterized by reproducibility contributions across the investigated frequency range, although X-MET shows a larger contribution from instrumentation and repeatability terms, particularly at low frequencies (e.g., 10 Hz). In contrast, SOL exhibits an uncertainty profile almost entirely dominated by reproducibility, with minimal instrumental and repeatability effects. Residual electrode–tissue interface effects may contribute to the increased uncertainty observed at low frequencies, although their impact is mitigated by the tetrapolar configuration. The proposed set-up did not allow for a quantitative separation between interface-related and intrinsic biological variability and will be addressed in future work.

For the dissipation factor, the variability structure differs substantially between the two materials. Native muscle exhibits a relatively stable relative uncertainty spectrum across the investigated frequency range, with a gradual increase at higher frequencies but without isolated instability

peaks. In contrast, X-MET shows moderate uncertainty levels in the low-to-intermediate frequency range, followed by a marked increase and isolated peaks in the kHz range, exceeding 60% at specific frequencies. These high-frequency fluctuations indicate a reduction in the metrological reliability of D estimation for X-MET. This suggests that measurements of D in this frequency region are not robust enough to be considered quantitatively reliable. Outside this high-frequency band, X-MET maintains acceptable uncertainty levels, comparable to those observed in native muscle. These results indicate that the uncertainty observed in X-MET arises from a combination of instrumentation and sample-dependent variability mechanisms, rather than from isolated measurement instability. The engineered construct therefore behaves as a complex system whose metrological reliability degrades specifically in the high-frequency regime, while remaining consistent with biological tissue behavior in the remaining frequency range. The analysis of relative uncertainty confirms the overall reliability of the measurements. Nevertheless, the impact of uncertainty is parameter-dependent. Previous bioimpedance studies on muscle tissue have reported variability levels of similar magnitude while still providing reliable and physiologically interpretable spectral responses, supporting the overall validity of the trends observed in the present work [61], [62], [63].

Finally, normalization of impedance magnitude further highlights the robustness of the engineered model. After removal of geometric scaling effects, X-MET retains the same qualitative frequency dependence observed in SOL, confirming that its electrical behavior is not merely geometry-driven. Normalization introduces only a very small increase in propagated uncertainty, particularly in X-MET, while the overall trends remain stable and clearly distinguishable, demonstrating the consistency of the underlying electrical response.

Overall, these findings show that uncertainty analysis represents a fundamental tool for quantifying measurement limitations and assessing the reliability of engineered tissues, rather than a direct validation of their intrinsic biological performance. Despite exhibiting larger uncertainty than native muscle, X-MET demonstrates reproducible and physically coherent electrical behavior across frequency, supporting its potential as a representative *in vitro* muscle model. The results further suggest that future improvements in fabrication uniformity and interface control may reduce uncertainty while preserving the biologically relevant variability that characterizes engineered living systems. From a wider perspective, the present study extends the role of uncertainty analysis beyond conventional error quantification. Previous uncertainty studies in impedance spectroscopy have mainly employed uncertainty to evaluate instrumentation performance, calibration procedures, or parameter estimation [30], [31]. In contrast, the proposed framework uses uncertainty as a metrological indicator of tissue reliability, enabling discrimination between intrinsic biological variability and measurement instability and identifying the frequency regions

in which engineered muscle can be considered electrically representative of native tissue.

V. CONCLUSION

This study analyzed the frequency-dependent uncertainty behavior of an engineered muscle system compared to a native muscle reference, showing that measurement reliability is governed by distinct frequency-dependent regimes rather than a uniform response across the spectrum. Despite exhibiting higher uncertainty than native muscle, X-MET demonstrates stable, reproducible, and physically coherent electrical behavior across most of the investigated frequency range. The source of uncertainties decomposition indicates that the observed uncertainty arises from distributed multiphysical variability rather than isolated measurement instability, supporting the interpretation of X-MET as a biologically representative and electrically consistent engineered tissue model. Moreover, the preservation of clear frequency-dependent impedance trends and acceptable uncertainties over the frequency regions characterized by bounded uncertainty levels confirms the robustness of the engineered construct despite its intrinsically more heterogeneous architecture. From a translational perspective, these findings support the potential of engineered muscle systems as reliable *in vitro* platforms for bioimpedance-based sensing and biomedical monitoring applications. More broadly, the present work demonstrates that uncertainty analysis can be employed as a metrological validation framework for engineered biological tissues. Rather than solely quantifying measurement limitations, it enables identification of frequency windows where impedance measurements remain electrically reliable and biologically interpretable, thereby supporting the translation of engineered muscle constructs toward bioimpedance-based sensing and monitoring applications.

REFERENCES

- [1] L. Horváth, M. Pekař, Z. Švagera, V. Horká, M. Mráz, and M. Bužga, "Skeletal muscle as an auto-, para- and endocrine organ: The role of myokines in muscle metabolism and other metabolic organs," *Physiological Res.*, vol. 2025, p. 37, Dec. 2025, doi: [10.33549/physiolres.935751](https://doi.org/10.33549/physiolres.935751).
- [2] Y. Yamada, B. Buehring, D. Krueger, R. M. Anderson, D. A. Schoeller, and N. Binkley, "Electrical properties assessed by bioelectrical impedance spectroscopy as biomarkers of age-related loss of skeletal muscle quantity and quality," *Journals Gerontology A, Biol. Sci. Med. Sci.*, vol. 72, no. 9, pp. 1180–1186, Nov. 2016, doi: [10.1093/gerona/glw225](https://doi.org/10.1093/gerona/glw225).
- [3] S. Jana, S. K. L. Levengood, and M. Zhang, "Anisotropic materials for skeletal-muscle-tissue engineering," *Adv. Mater.*, vol. 28, no. 48, pp. 10588–10612, Dec. 2016, doi: [10.1002/adma.201600240](https://doi.org/10.1002/adma.201600240).
- [4] T. Cao and C. R. Warren, "From 2D myotube cultures to 3D engineered skeletal muscle constructs: A comprehensive review of *in vitro* skeletal muscle models and disease modeling applications," *Cells*, vol. 14, no. 12, p. 882, Jun. 2025, doi: [10.3390/cells14120882](https://doi.org/10.3390/cells14120882).
- [5] M. Volpi, A. Paradiso, M. Costantini, and W. Świe szkowski, "Hydrogel-based fiber biofabrication techniques for skeletal muscle tissue engineering," *ACS Biomaterials Sci. Eng.*, vol. 8, no. 2, pp. 379–405, Feb. 2022, doi: [10.1021/acsbmaterials.1c01145](https://doi.org/10.1021/acsbmaterials.1c01145).
- [6] T. Fan et al., "Engineering strategies for the construction of oriented and functional skeletal muscle tissues," *Biofabrication*, vol. 17, no. 2, Apr. 2025, Art. no. 022013, doi: [10.1088/1758-5090/adbfc2](https://doi.org/10.1088/1758-5090/adbfc2).

- [7] A. A. Appel, M. A. Anastasio, J. C. Larson, and E. M. Brey, "Imaging challenges in biomaterials and tissue engineering," *Biomaterials*, vol. 34, no. 28, pp. 6615–6630, Sep. 2013, doi: [10.1016/j.biomaterials.2013.05.033](#).
- [8] S. Y. Nam, L. M. Ricles, L. J. Suggs, and S. Y. Emelianov, "Imaging strategies for tissue engineering applications," *Tissue Eng. B, Rev.*, vol. 21, no. 1, pp. 88–102, Feb. 2015, doi: [10.1089/ten.teb.2014.0180](#).
- [9] N. J. Castro, G. Babakhanova, J. Hu, and K. A. Athanasiou, "Nondestructive testing of native and tissue-engineered medical products: Adding numbers to pictures," *Trends Biotechnol.*, vol. 40, no. 2, pp. 194–209, Feb. 2022, doi: [10.1016/j.tibtech.2021.06.009](#).
- [10] A. K. Haudenschild et al., "Non-destructive, continuous monitoring of biochemical, mechanical, and structural maturation in engineered tissue," *Sci. Rep.*, vol. 12, no. 1, p. 16227, Sep. 2022, doi: [10.1038/s41598-022-18702-x](#).
- [11] K. P. Quinn, E. Bellas, N. Fourligas, K. Lee, D. L. Kaplan, and I. Georgakoudi, "Characterization of metabolic changes associated with the functional development of 3D engineered tissues by non-invasive, dynamic measurement of individual cell redox ratios," *Biomaterials*, vol. 33, no. 21, pp. 5341–5348, Jul. 2012, doi: [10.1016/j.biomaterials.2012.04.024](#).
- [12] T. K. Bera, "Bioelectrical impedance methods for noninvasive health monitoring: A review," *J. Med. Eng.*, vol. 2014, pp. 1–28, Jun. 2014, doi: [10.1155/2014/381251](#).
- [13] D. A. Dean, T. Ramanathan, D. Machado, and R. Sundararajan, "Electrical impedance spectroscopy study of biological tissues," *J. Electrostatics*, vol. 66, nos. 3–4, pp. 165–177, Mar. 2008, doi: [10.1016/j.jelstat.2007.11.005](#).
- [14] J. A. Nagy, C. J. DiDonato, S. B. Rutkove, and B. Sanchez, "Permittivity of ex vivo healthy and diseased murine skeletal muscle from 10 kHz to 1 MHz," *Sci. Data*, vol. 6, no. 1, p. 37, Apr. 2019, doi: [10.1038/s41597-019-0045-2](#).
- [15] B. Sanchez and S. B. Rutkove, "Electrical impedance myography and its applications in neuromuscular disorders," *Neurotherapeutics*, vol. 14, no. 1, pp. 107–118, Jan. 2017, doi: [10.1007/s13311-016-0491-x](#).
- [16] M. Amini, J. Hisdal, and H. Kalvøy, "Applications of bioimpedance measurement techniques in tissue engineering," *J. Electr. Bioimpedance*, vol. 9, no. 1, pp. 142–158, Dec. 2018, doi: [10.2478/joeb-2018-0019](#).
- [17] K. Asami. (2002). *Characterization of Heterogeneous Systems By Dielectric Spectroscopy*. [Online]. Available: <https://www.elsevier.com/locate/ppolysci>
- [18] D. Miklavčič et al., "Electric properties of tissues," in *Wiley Encyclopedia of Biomedical Engineering*. Hoboken, NJ, USA: Wiley, 2006.
- [19] H. P. Schwan, "Electrical properties of tissue and cell suspensions," in *Advances in Biological and Medical Physics*, 1957, doi: [10.1016/B978-1-4832-3111-2.50008-0](#).
- [20] S. Abasi, J. R. Aggas, G. G. Garayar-Leyva, B. K. Walther, and A. Guiseppi-Elie, "Bioelectrical impedance spectroscopy for monitoring mammalian cells and tissues under different frequency domains: A review," *ACS Meas. Sci. Au*, vol. 2, no. 6, pp. 495–516, Dec. 2022, doi: [10.1021/acsmesuresciau.2c00033](#).
- [21] Y. Akamatsu et al., "Phase angle from bioelectrical impedance analysis is a useful indicator of muscle quality," *J. Cachexia, Sarcopenia Muscle*, vol. 13, no. 1, pp. 180–189, Feb. 2022, doi: [10.1002/jcsm.12860](#).
- [22] G. Gafarov et al., "Determination of intracellular electrical parameters in bioelectrical impedance analysis," in *Proc. Web Conf.*, 2023, pp. 1–11, doi: [10.1051/bioconf/20237101114](#).
- [23] Y. Yamada et al., "Phase angle obtained via bioelectrical impedance analysis and objectively measured physical activity or exercise habits," *Sci. Rep.*, vol. 12, no. 1, p. 17274, Oct. 2022, doi: [10.1038/s41598-022-21095-6](#).
- [24] S. Carosio, L. Barberi, E. Rizzuto, C. Nicoletti, Z. D. Prete, and A. Musarò, "Generation of ex vivo-vascularized muscle engineered tissue (X-MET)," *Sci. Rep.*, vol. 3, no. 1, p. 1420, Mar. 2013, doi: [10.1038/srep01420](#).
- [25] M. Cosentino et al., "Remodeled ex vivo muscle engineered tissue improves heart function after chronic myocardial ischemia," *Sci. Rep.*, vol. 13, no. 1, p. 10370, Jun. 2023, doi: [10.1038/s41598-023-37553-8](#).
- [26] Z. Del Prete, A. Musarò, and E. Rizzuto, "Measuring mechanical properties, including isotonic fatigue, of fast and slow MLC/mIgf-1 transgenic skeletal muscle," *Ann. Biomed. Eng.*, vol. 36, no. 7, pp. 1281–1290, Jul. 2008, doi: [10.1007/s10439-008-9496-x](#).
- [27] S. Pisu, M. Cosentino, L. Apa, A. Musarò, E. Rizzuto, and Z. D. Prete, "Measuring the maximum power of an ex vivo engineered muscle tissue with isovelocity shortening technique," *IEEE Trans. Instrum. Meas.*, vol. 68, no. 7, pp. 2404–2411, Jul. 2019, doi: [10.1109/TIM.2019.2904071](#).
- [28] S. Pisu, L. Apa, M. Cosentino, A. Musarò, E. Rizzuto, and Z. Del prete, "Measuring the X-MET's maximum power: A preliminary study," in *Proc. IEEE Int. Symp. Med. Meas. Appl. (MeMeA)*, Jun. 2018, pp. 1–5.
- [29] M. Ingresso et al., "Electrical signature of engineered muscle tissue: Multi-frequency impedance analysis of X-MET," in *Proc. IEEE Med. Meas. Appl. (MeMeA)*, Apr. 2026, pp. 1–6, doi: [10.1109/MEMEA69746.2026.11537289](#).
- [30] V. Vivier and M. E. Orazem, "Impedance analysis of electrochemical systems," *Chem. Rev.*, vol. 122, no. 12, pp. 11131–11168, Jun. 2022, doi: [10.1021/acs.chemrev.1c00876](#).
- [31] A. Moradpour, M. Kasper, J. Hoffmann, and F. Kienberger, "Measurement uncertainty in battery electrochemical impedance spectroscopy," *IEEE Trans. Instrum. Meas.*, vol. 71, pp. 1–9, 2022, doi: [10.1109/TIM.2022.3198466](#).
- [32] N. B. Roozen and E. A. Piana, "Uncertainty analysis of acoustic and non-acoustic parameters derived from four-microphone impedance tube measurements," *Appl. Acoust.*, vol. 198, Sep. 2022, Art. no. 109002, doi: [10.1016/j.apacoust.2022.109002](#).
- [33] L. A. Q. Blandón, J. Ramón-Azcón, M. R. Gutiérrez, D. A. Garzón-Alvarado, and J. J. Vaca-González, "Dielectric characterization of skeletal muscle cell culture medium using electrochemical impedance spectroscopy," *Electroanalysis*, vol. 37, no. 10, Oct. 2025, Art. no. e70069, doi: [10.1002/elan.70069](#).
- [34] A. Bailleul et al., "In vitro impedance spectroscopy: A MEA-based measurement bench for myoblasts cultures monitoring," in *Proc. XXXVI Conf. Design Circuits Integr. Syst. (DCIS)*, Nov. 2021, pp. 1–6, doi: [10.1109/DCIS53048.2021.9666172](#).
- [35] A. Bailleul et al., "Bioimpedance spectroscopy helps monitor the impact of electrical stimulation on muscle cells," *IEEE Access*, vol. 10, pp. 131430–131441, 2022, doi: [10.1109/ACCESS.2022.3228479](#).
- [36] K. Goyal, D. Shah, and S. W. Day, "Day-to-day variability in measurements of respiration using bioimpedance from a non-standard location," *Sensors*, vol. 24, no. 14, p. 4612, Jul. 2024, doi: [10.3390/s24144612](#).
- [37] A. Iftime, C. Scheau, R.-M. Babeş, D. Ionescu, A. Periferakis, and O. Călinescu, "Confounding factors and their mitigation in measurements of bioelectrical impedance at the skin interface," *Bioengineering*, vol. 12, no. 9, p. 926, Aug. 2025, doi: [10.3390/bioengineering12090926](#).
- [38] C. Gabriel, S. Gabriel, and E. Corthout, "The dielectric properties of biological tissues: I. Literature survey," *Phys. Med. Biol.*, vol. 41, no. 11, pp. 2231–2249, Nov. 1996, doi: [10.1088/0031-9155/41/11/001](#).
- [39] C. Jönsson, K. Johansson, M. Bjurberg, and C. Brogårdh, "Impedance of extracellular fluid, volume, and local tissue water can be reliably measured in people with lower limb lymphedema," *Phys. Therapy*, vol. 102, no. 5, May 2022, Art. no. pzac025, doi: [10.1093/ptj/pzac025](#).
- [40] D. P. Looney et al., "Reliability, biological variability, and accuracy of multi-frequency bioelectrical impedance analysis for measuring body composition components," *Frontiers Nutrition*, vol. 11, Dec. 2024, Art. no. 1491931, doi: [10.3389/fnut.2024.1491931](#).
- [41] V. Garr Barry, J. L. Chiang, K. G. Bowman, K. D. Johnson, and B. A. Gower, "Bioimpedance-derived membrane capacitance: Clinically relevant sources of variability, precision, and reliability," *Int. J. Environ. Res. Public Health*, vol. 20, no. 1, p. 686, Dec. 2022, doi: [10.3390/ijerph20010686](#).
- [42] C. Leys, C. Ley, O. Klein, P. Bernard, and L. Licata, "Detecting outliers: Do not use standard deviation around the mean, use absolute deviation around the median," *J. Experim. Social Psychol.*, vol. 49, no. 4, pp. 764–766, Jul. 2013, doi: [10.1016/j.jesp.2013.03.013](#).
- [43] S. Afzal, A. Afzal, M. Amin, S. Saleem, N. Ali, and M. Sajid, "A novel approach for outlier detection in multivariate data," *Math. Problems Eng.*, vol. 2021, pp. 1–12, Oct. 2021, doi: [10.1155/2021/1899225](#).
- [44] V. Aschepkov, "Methods for outlier detection in metrological studies," *Measuring Equip. Metrology*, vol. 85, no. 3, pp. 25–29, 2024, doi: [10.23939/istcmtm2024.03.025](#).
- [45] B. Voloh, M. R. Watson, S. König, and T. Womelsdorf, "MAD saccade: Statistically robust saccade threshold estimation via the median absolute deviation," *J. Eye Movement Res.*, vol. 12, no. 8, p. 65, May 2020, doi: [10.16910/jemr.12.8.3](#).

- [46] LCR Meter LCR-6300/6200/6100/6020/6002 User's Guide VERSION: 1.07, Standard ISO-9001, 2020.
- [47] P. Arpaia, F. Mancino, and N. Moccaldi, "A reproducible bioimpedance transducer for insulin noninvasive measurement," *IEEE Trans. Instrum. Meas.*, vol. 72, pp. 1–11, 2023, doi: [10.1109/TIM.2023.3264037](https://doi.org/10.1109/TIM.2023.3264037).
- [48] I. Farrance and R. Frenkel, "Uncertainty of measurement: A review of the rules for calculating uncertainty components through functional relationships," *Clin. Biochem. Rev.*, vol. 33, no. 2, p. 49, 2012.
- [49] J. Zhu, Y. Wang, K. Zhao, Y. Wang, C. Fu, and K. Man, "Assessment of measurement uncertainty for S-parameter measurement based on covariance matrix," *Sensors*, vol. 24, no. 11, p. 3668, Jun. 2024, doi: [10.3390/s24113668](https://doi.org/10.3390/s24113668).
- [50] A. Prasad and M. Roy, "Bioimpedance analysis of vascular tissue and fluid flow in human and plant body: A review," *Biosystems Eng.*, vol. 197, pp. 170–187, Sep. 2020, doi: [10.1016/j.biosystemseng.2020.06.006](https://doi.org/10.1016/j.biosystemseng.2020.06.006).
- [51] E. Lancia, D. Marazzi, L. Apa, F. Trovalusci, Z. D. Prete, and E. Rizzuto, "Evaluation of the uncertainty associated with the young's modulus measurement of 3-D-printed specimens for intracranial aneurysm mock-up realization," *IEEE Trans. Instrum. Meas.*, vol. 75, 2026, Art. no. 1004915, doi: [10.1109/TIM.2026.3697084](https://doi.org/10.1109/TIM.2026.3697084).
- [52] L. Apa, L. D'Alvia, Z. D. Prete, and E. Rizzuto, "A characterization of the uncertainties associated with an automated system for the study of lithium-ion cells: A case-study of a domestic grid 24-h scenario," *IEEE Trans. Instrum. Meas.*, vol. 73, pp. 1–11, 2024, doi: [10.1109/TIM.2024.3476559](https://doi.org/10.1109/TIM.2024.3476559).
- [53] JCGM. (2008). *Evaluation of Measurement Data-Guide to the Expression of Uncertainty in Measurement* [Internet]. [Online]. Available: <https://www.bipm.org>
- [54] J.-L. Bertrand-Krajewski, M. Uhl, and F. H. L. R. Clemens-Meyer, "Uncertainty assessment," in *Metrology in Urban Drainage and Stormwater Management: Plug and Pray*. U.K.: IWA Publishing, 2021, pp. 263–326, doi: [10.2166/9781789060119_0263](https://doi.org/10.2166/9781789060119_0263).
- [55] K. Agarwal et al., "Uncertainty analysis of Atlantic salmon fish scale's acoustic impedance using 30 MHz C-scan measurements," *Ultrasonics*, vol. 142, Aug. 2024, Art. no. 107360, doi: [10.1016/j.ultras.2024.107360](https://doi.org/10.1016/j.ultras.2024.107360).
- [56] U. Baysal and B. M. Eyüboğlu, "Tissue resistivity estimation in the presence of positional and geometrical uncertainties," *Phys. Med. Biol.*, vol. 45, no. 8, pp. 2373–2388, Aug. 2000.
- [57] M. Bilušić and L. Olivari, "Assessment of process chain suitability of the optical 3D measuring system by using influencing factors for measurement uncertainty," *J. Ind. Inf. Integr.*, vol. 41, Sep. 2024, Art. no. 100654, doi: [10.1016/j.jii.2024.100654](https://doi.org/10.1016/j.jii.2024.100654).
- [58] N. Senin, S. Catalucci, M. Moretti, and R. K. Leach, "Statistical point cloud model to investigate measurement uncertainty in coordinate metrology," *Precis. Eng.*, vol. 70, pp. 44–62, Jul. 2021, doi: [10.1016/j.precisioneng.2021.01.008](https://doi.org/10.1016/j.precisioneng.2021.01.008).
- [59] A. Kallner and E. Theodorsson, "An experimental study of methods for the analysis of variance components in the inference of laboratory information," *Scandin. J. Clin. Lab. Invest.*, vol. 80, no. 1, pp. 73–80, Jan. 2020, doi: [10.1080/00365513.2019.1700426](https://doi.org/10.1080/00365513.2019.1700426).
- [60] M. Ingresso et al., "Determination of the uncertainties associated with the measurement of the main parameters characterizing mouse uterosacral ligament quasi-static behavior," *IEEE Open J. Instrum. Meas.*, vol. 4, pp. 1–12, Oct. 2025, doi: [10.1109/OJIM.2025.3626110](https://doi.org/10.1109/OJIM.2025.3626110).
- [61] M. Martinez-Gonzalez, J. Montilla-Herrador, J. A. García-Vidal, P. Escobar-Reina, M. Gacto-Sánchez, and F. Medina-Mirapeix, "Intra- and inter-rater reliability of electrical impedance myography using adhesive electrodes in healthy volunteers," *J. Electromyogr. Kinesiol.*, vol. 55, Dec. 2020, Art. no. 102456, doi: [10.1016/j.jelekin.2020.102456](https://doi.org/10.1016/j.jelekin.2020.102456).
- [62] A. Sawant et al., "Reliability of calf bioelectrical impedance spectroscopy and magnetic-resonance-imaging-acquired skeletal muscle hydration measures in healthy people," *Physiol. J.*, vol. 2013, pp. 1–12, Sep. 2013, doi: [10.1155/2013/563494](https://doi.org/10.1155/2013/563494).
- [63] R. D. C. Honorato, A. S. M. Ferraz, W. Kassiano, D. P. Carvalho, and V. M. Ceccatto, "Test-retest reliability of electrical impedance myography in hamstrings of healthy young men," *J. Electromyogr. Kinesiol.*, vol. 56, Feb. 2021, Art. no. 102511, doi: [10.1016/j.jelekin.2020.102511](https://doi.org/10.1016/j.jelekin.2020.102511).