Design of Bioimpedance Spectroscopy Instrument With Compensation Techniques for Soft Tissue Characterization

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Bioimpedance spectroscopy (BIS) has shown significant potential in many areas of medicine to provide new physiologic markers. Several acute and chronic diseases are accompanied by changes in intra- and extracellular fluid within various areas of the human body. The estimation of fluid in various body compartments is therefore a simple and convenient method to monitor certain disease states. In this work, the design and evaluation of a BIS instrument are presented and three key areas of the development process investigated facilitating the BIS measurement of tissue hydration state. First, the benefit of incorporating DC-stabilizing circuitry to the standard modified Howland current pump (MHCP) is investigated to minimize the effect of DC offsets limiting the dynamic range of the system. Second, the influence of the distance between the bioimpedance probe and a high impedance material is investigated using finite element analysis (FEA). Third, an analytic compensation technique is presented to minimize the influence of parasitic capacitance. Finally, the overall experimental setup is evaluated through ex vivo BIS measurements of porcine spleen tissue and compared to published results. The DC-stabilizing circuit demonstrated its ability to maintain DC offsets at less than 650 μV through 100 kHz while maintaining an output impedance of 1 $\text{M}\Omega$ from 100 Hz to 100 kHz. The proximity of a bioimpedance probe to a high impedance material such as acrylic was shown to increase measured impedance readings by a factor of 4x as the ratio of the distance between the sensing electrodes to the distance between the bioimpedance probe and acrylic reached 1:3. The average parasitic capacitance for the circuit presented was found to be 712 ± 128 pF, and the analytic compensation method was shown to be able to minimize this effect on the BIS measurements. Measurements of porcine spleen tissue showed close correlation with experimental results reported in published articles. This research presents the successful design and evaluation of a BIS instrument. Specifically, robust measurements were obtained by implementing a DC-stabilized current source, investigating probe-material proximity issues and compensating for parasitic capacitance. These strategies were shown to provide tissue measurements comparable with published literature. [DOI: 10.1115/1.4029706]

Keywords: bioimpedance, spectroscopy, current source, soft-tissue

1 Introduction

Bioimpedance measurements have found broad applications in the analysis and monitoring of numerous physiological processes and are of importance to researchers, physiologists, and clinicians [1]. They have been used in many areas of clinical care to measure physiological events, for example, heart stroke and respiration volume, edema, urological tissue characterization, myography, body composition analysis, and electroporation monitoring [2]. The complex electrical impedance measurements obtained can be utilized for noninvasive tissue characterization since tissue composition, structures, and health status influence the measurements [3].

BIS is generally preferred over the traditional single, fixed frequency bioimpedance analysis methods, since it provides a more comprehensive interrogation of the tissue. Researchers have investigated applications of BIS for intra-, extracellular and body water measurement [4], hydration monitoring to guide dialysis care in chronic kidney disease patients [5], total body water monitoring for chronic obstructive pulmonary disease patients [6], and

to monitor the clinical progress of heart failure patients using thoracic electrical bioimpedance cardiac performance measurements [7]. In more basic research, BIS has been used to characterize tissue samples, for example, Karki et al. performed BIS measurements of ex vivo muscle tissue and demonstrated the expected higher anisotropy of muscle tissue compared to liver tissue and obtained good agreement with published literature [8]. Chauveau et al. [9], performed ex vivo bioimpedance measurements on normal and cancerous female breast tissue biopsies and showed that the electrical properties of normal tissues, surrounding tissues, and carcinoma were different. Nescolarde et al. [10] investigated BIS for muscle injury assessment, while Hernandez et al. [11] studied BIS for percutaneous tissue access. Among other research areas, bioimpedance monitoring has led to the development of electrical impedance tomography, where tissue volumes are mapped to provide rudimentary visualization [12–14].

Common issues encountered during the development of bioimpedance systems include variability in the contact impedance between the electrodes and tissue sample, heterogeneity of the measurement space either in vivo or on the benchtop, and stray capacitance concerns. In order to mitigate these issues, careful design and robust compensation techniques must be employed during the development of the system to obtain reliably, high quality BIS measurements that are useful for further analysis.

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This manuscript investigates the implementation of compensation techniques used to optimize BIS measurements. First, on the hardware side, the design, implementation, and evaluation of a MHCP utilizing a DC stabilizing technique to overcome dynamic range limitations seen in noncompensated MHCP designs is discussed. Second, on the experimental side, the effect of the experimental setup on measured bioimpedance readings is discussed. Third, for postacquisition digital signal processing, a numerical method for stray capacitance compensation is discussed. The utility of these compensation strategies is demonstrated experimentally by obtaining bioimpedance measurements from ex vivo porcine spleen samples and comparing the results to previously reported literature values.

2 Background

Measuring tissue impedance at a particular frequency requires an alternating electrical current at that frequency to be passed through the section of tissue under investigation [3]. To obtain the complex impedance, a differential measurement of the voltage magnitude and phase of the signal between the source and sensing electrodes is performed and compared to the source signal [2]. Biological tissues are frequency-dependent materials, and by performing a frequency sweep, resistance, and reactance measurements can be recorded on a Cartesian plot, ideally revealing a semicircular locus describing the electrical properties of the tissue volume under investigation.

The experimentally derived Cole equation provides one method which can be used to interpolate the measured values, and obtain key constants describing the tissue sample [15]. The electrical circuit representation of the Cole equation is comprised of two parallel paths, one comprising a resistive and the other a resistance with capacitive reactance (resistor–capacitor combination) path. The path containing the resistance alone represents the extracellular fluid component of tissue, while the path containing the reactance represents intracellular fluid, with the capacitance representing a dielectric effect due to cell walls [16]. BIS therefore provides the fundamental complex impedance measurements necessary to obtain a bulk estimate of the intra- and extracellular fluid resistances, and cell membrane capacitance by monitoring the impedance over a wide range of frequencies.

For the development of our BIS instrument, a linear tetrapolar bioimpedance electrode was developed to perform the experiments for this research, shown in Fig. 1. The cylindrical probe housing was machined from acrylic with a 19 mm diameter flat tissue contact area. The surface contacting the tissue had four flat, circular 0.25 mm diameter press-fit platinum electrodes that were centered and mounted flush with the surface of the housing. Each electrode was spaced 3 mm from the next electrode. The two outer electrodes were used to apply the source current, while the inner two sensing electrodes measured the potential difference. However, before the BIS circuit to interface with the probe could be designed, a knowledge of the measurand was necessary, which

Fig. 1 BIS probe prototype showing (a) actual probe and electrodes and (b) illustration of probe measuring tissue sample

primarily consists of the tissue impedance and electrode contact impedance. Since we planned to use porcine spleen tissue to evaluate the operation of the system, we developed an equivalent circuit based on the Cole function. Each of the model terms were estimated based on published data for spleen bioimpedance from Ref. [17], and determination of the BIS probe constant, k [18]. For a symmetric probe with flush electrodes in a semi-infinite medium, the probe constant is provided by Eq. (1) [19]. Where k is the probe constant in units of m^{-1} and l is the distance between the current carrying electrodes. The probe constant accounts for the geometry of the bioimpedance probe and is used to convert bioimpedance measurements to material electrical properties [20]. Considering l is 0.009 m for the bioimpedance probe used in this study, k is equal to 53 m^{-1}

$$k = \frac{3}{2\pi l} \quad (1)$$

Additionally, the contact impedance between the electrodes and tissue can be modeled as a series resistance and capacitor as was done by Schwan [21]. There are many other and more complex ways to model electrode contact effects, however, this method provides a simplified approach allowing the rapid estimation of this effect to guide the system design. Using this simplified method provides impedance values that can be estimated based on knowledge of the contact area and the assumption that contact impedance is proportional to surface area. A summary of the estimated impedances encountered by a bioimpedance circuit is shown in Fig. 2. The overall impedance, Z_{tot} , is the sum of the tissue impedance, Z_{tis} , and the contact impedance, Z_p . Z_{tot} is dominated at low frequencies by Z_p and by Z_{tis} at high frequencies. Z_{tis} is estimated to be 2 k Ω at 100 Hz and 1 k Ω at 100 kHz with a critical frequency at 7.5 kHz. Z_p is estimated to be 25 k Ω at 100 Hz and drops off to 150 Ω by 100 kHz.

3 System Design

The source current, output impedance, frequency response, and DC offsets (0 Hz signal components) are some key areas that require attention when designing or evaluating a BIS current source. There are several factors that tend to favor the use of a constant current source over a constant voltage source (VS) in driving a load (tissue) for bioimpedance measurements. Since tissue properties and contact impedances between the electrodes and tissue surface are highly frequency-dependent, and in series with the tissue being tested, a constant current source ensures a known,

Fig. 2 Expected impedance magnitudes to be driven by the current source based on literature values for platinum electrode contact impedance and the measured bioimpedance of spleen tissue

controlled current density passes through the tissue [21]. This is not only important for electrical safety but also to prevent any current induced tissue heating effects during the measurement process [22]. It has also been shown that a constant current source approach is beneficial in minimizing unwanted electrochemical reactions with the tissue due to low frequency electrode polarization [23,24].

A critical factor in any current source design is its output impedance, which corresponds to how large of a load the current source is able to drive. Since the output impedance of the current source is in parallel with the load (electrode and tissue impedances), the source current will be split along these two possible paths (excluding parasitic effects). For example, assuming a 10% maximum error in output current is specified, the output impedance would need to be greater than or equal to 10x the load impedance. However, since the load impedance of tissue is frequency-dependent, the current source must deliver a constant current through a wide range of frequencies, while maintaining a suitable output to load impedance ratio. Design configurations examined for bioimpedance measurements have included the use of current mirrors, MHCPs, and current conveyors [23,25–33].

Since BIS relies on varying the frequency of the current source, any DC component is undesirable in the output signal as it would result in additional power dissipation in the tissue, possibly resulting in added heating, as well as a DC offset in the measured voltage signal reducing the dynamic range of the system. The output signal can therefore be capacitively coupled to prevent any DC current entering the tissue. However, considering the ideal high output impedance of the output circuit, even low DC-offset currents can absorb much of the dynamic range due to the series output coupling capacitances. For example, if a voltage controlled current source (VCCS) was used where a 5 mV DC offset on the input reference resulted in a 5 μ A constant output current, for a 1 M Ω output impedance, the output voltage would have to increase to 5 V DC to pass the DC current through the output impedance. Depending on the power supply voltages, this may severely limit the magnitude of any superimposed sinusoidal output current signal. Obviously, methods to minimize any DC current in the output of the current source would be highly beneficial for a high-performance BIS instrument.

3.1 Compensated VCCS Design. To maintain a low-current density, a maximum output of 60 μ A RMS (root mean square) is specified. Using the electrode that was designed, results in a current density at the electrode–tissue interface of 1.18 mA/mm², which should not be exceeded over the required frequency range of 100 Hz to 100 kHz [18]. To achieve this, a VCCS design was used based on a MHCP. Figure 3 provides the final circuit design used in this study. The standard transfer function for the output current of a MHCP in our configuration can be shown to reduce down to Eq. (2) as described by Stitt [34]. Where v_1 and v_2 are the voltages provided to the noninverting and inverting inputs, respectively (AMP03 in Fig. 3), and R represents the output resistor and is used to set the output current of the circuit

$$i_o = \frac{(v_1 - v_2)}{R} \quad (2)$$

The VCCS design was based on the AMP03 differential amplifier as the central component based on its matched network of 25 k Ω internal resistors, bandwidth of 3 MHz, and fast 9.5 V/ μ s slew rate. The AD8065 operational amplifier was chosen to isolate the current sense resistor from the feedback loop of the AMP03 for low-current stability as well as prevent input bias currents from the AMP03 effecting the output current. The output signal from the AD8065 amplifier is also used to drive the coaxial cable shield, thus reducing stray capacitance from the coaxial cable [35,36]. However, DC offset voltages from the output of the AD8065 can lead to a DC offset of the current at the output of the

AMP03. To overcome this limitation and compensate for the offset voltage of the AD8065, an additional DC stabilization circuit using an LM7171 amplifier was used [23,25]. The circuit was designed as a low-pass filter with a 3 dB cut-off frequency of 0.16 Hz. The capacitive reactance in the feedback loop additionally minimizes the gain at higher frequencies and provides an almost open-loop gain to DC signals. The DC stabilization circuit was incorporated as a feedback element into the inverting input of the AMP03, effectively providing a voltage bias to the v_2 input of the modified Howland transfer function to minimize DC offsets. Selection criteria for this amplifier included a high open-loop voltage gain, high common mode rejection ratio (CMRR), and low DC offset voltage.

3.2 Measurement Automation and Acquisition. An Agilent 33120A function generator (Agilent Technologies, Santa Clara, CA) provided a sinusoidal VS to control the VCCS, which in turn provides a controlled current to the tissue sample. Voltage readings across the two sense electrodes are first buffered by an AD8065 operational amplifiers connected in voltage follower configurations after which their outputs are differentially amplified using an INA111 instrumentation amplifier (IA) with a voltage gain of 10. The output from the buffer stage is also used to drive the coaxial shield from the BNC extensions of the bioimpedance probe to minimize any capacitance introduced by the cable. The output from the IA is then passed through an LM7171 operational amplifier in an inverting configuration with a voltage gain of -2 for a total gain of -20 . The output of this stage is sent to a PXI-5044 oscilloscope (National Instruments, Austin, TX) for data acquisition. Current output was measured via a current-to-voltage converter (C2V) circuit using a LM7171 with a gain of 20,000 V/A. The output of this stage is also sent to the PXI-5044 oscilloscope for data acquisition. A Labview® (National Instruments, Austin, TX) program controlled the function generator and oscilloscope to sweep the frequency over the intended range and acquire the resulting measurements.

4 Methods

After the experimental system was assembled, various aspects of the system were studied to ensure reliable data could be obtained.

4.1 Output Impedance Analysis. Equation (3) provides the theoretical output impedance of the MHCP as described in Stitt [34]. Where, Z_o is the equivalent output impedance of the current source, measured in ohms, $c(\omega)$ represents the frequency-dependent CMRR of the output amplifier (dB), and R_x is the parallel combination of the external current sense resistor, R , and 25 k Ω

$$Z_o = R_x \cdot 10^{\frac{c(\omega)}{20}} \quad (3)$$

Both the standard and DC-stabilized MHCP circuits were also simulated using Spice (Multisim, National Instruments, Austin, TX), and the DC offset and output impedance measured. Simulating the circuit provided an ideal benchmark against which we could compare the circuits, our experimental measurements, and it provided a way to fully characterize the ideal circuit performance without extensive experimentation. Since a Spice model for the AMP03 was not available, a custom model was created using the frequency-dependent CMRR of the Analog Devices OP282 operational amplifier model, setup in a differential amplifier configuration with 25 k Ω resistors as recommended by Analog Devices technical support [37]. The resistors used to create the differential amplifier were given 0.002% tolerances per the AMP03 datasheet. The frequency-dependent CMRR was obtained directly from the OP282 datasheet.

Fig. 3 Circuit diagram with experimental setup for the measurement system. The function generator supplies the reference voltage for the current source whose output is measured by the current-to-voltage converter. The differential amplifier uses a front end (AD8065) with very high input impedance to sense the voltage difference between the inner two electrodes and DC couples the readings. The output from the differential amplifier and the current-to-voltage converter are sent to an oscilloscope for measurement.

In addition, the actual output impedance of the physical VCCS circuit was measured by driving two different loads with the current source. In this way, the output impedance can be empirically determined using Eq. (4) [38]. Where ΔR_L is the difference between the two loads, ΔI is the measured current difference between the two loads, and I is the intended current. Loads of 100 Ω and 1 k Ω were used for the output impedance characterization

$$|Z| \cong \frac{\Delta R_L I}{\Delta I} \quad (4)$$

4.2 Stray Capacitance. Stray capacitance is a known concern when performing bioimpedance measurements [39–41]. It can be analytically described as a capacitor placed in parallel with the series combination of the electrode impedance and material being tested. Stray capacitance results in a characteristic deviation in the measurement readings commonly referred to as a hook, or tail, effect most noticeable at higher frequencies. Sources of stray capacitance include the coaxial cables connecting the bioimpedance circuit to the BIS probe, BIS probe contact–material contact interface, traces on the bioimpedance circuit board, and routes to ground through the experimental setup.

Various methods for dealing with stray capacitance have been presented in the literature, the most common of which is the time-

delay (Td) compensation technique [42]. Recently, an improvement to this method has been proposed allowing for compensation of both the modulus and phase changes as a result of the presence of a stray capacitance [35,40,43]. This method takes advantage of the accepted view of tissue as a single dispersion system [16]. In such systems, the susceptance tends to zero as the output frequency increases.

The methodology of Buendia et al. [43] for removing the effect of stray capacitance on bioimpedance measurements was used for this investigation. This technique is similar to those more commonly used, such as the Td method, but allows for compensation of both the modulus and phase of the measured data due to stray capacitance. Viewing stray capacitance as a capacitor in parallel with the tissue, the amount of leakage current through this capacitance naturally increases with frequency and, therefore, so will the overall susceptance of the measurement. Due to the negligible susceptance ($\text{Im}[Y]$, where $Y = 1/Z$) of tissue at high frequencies, the stray capacitance (C_{par}) can be estimated by the slope of the graph of susceptance plotted against natural frequency, ω , where $\omega = 2\pi f$. A conditioned impedance reading can then be calculated using Eq. (5) [43]. Where Z_{cond} is the resulting conditioned impedance measurement, Z_{meas} is the measured impedance value, ω is the natural frequency, C_{par} is the parasitic capacitance, and j is $\sqrt{-1}$

$$Z_{\text{cond}}(\omega) = Z_{\text{meas}}(\omega)e^{-\log(1-j\omega Z_{\text{meas}}(\omega)C_{\text{par}})} \quad (5)$$

4.3 Influence of Measurement Volume Geometry. When tissue undergoes dynamic changes, such as when it is put into a test chamber or placed under strain, the bioimpedance probe may get closer to a material interface which has a much higher impedance than the tissue (i.e., acrylic and bone). It has previously been shown that impedance measurements are dependent on the distance of the probe to other dissimilar materials, especially as the change in impedance between differing layers increases [44–46]. This effect is unavoidable in vivo unless some knowledge of the underlying tissue composition is available. Based on this knowledge, models can be formulated to compensate for this effect; however, this discussion is beyond the scope of this research. For this research, we investigate this effect in a controlled environment utilizing FEA and empirical experiments within an acrylic tissue chamber to study and inform the reader of this phenomenon.

To determine how the proximity of the bioimpedance probe to the bottom of the acrylic tissue chamber affects the measured bioimpedance readings, a FEA of the experimental setup was performed parameterizing the probe depth using COMSOL Multiphysics (Boston, MA). The FEA simulated the placement of the two source and two sensing electrodes 0.25 mm in diameter with 3 mm interelectrode spacing on the surface of a 25.4 mm diameter cylinder of saline solution. The 3D model is shown in Fig. 4. All surfaces except for the tops of the outer two electrodes were given an insulating boundary condition. One outer electrode was given a normal current density of 1.184 mA/mm² to simulate the injection of 60 μ A of current and the opposite electrode served as the return electrode. A quasi-static electrical conduction model was applied to solve for the electric field in the saline solution using the Laplace equation shown in Eq. (6). Where V is the electric potential and σ is the electrical conductivity of the saline. The height of the saline solution (h) was increased until the calculated potential difference between the two center electrodes was less than 1 mV. This point served as the reference for a semi-infinite depth of saline and h was decreased down to 1 mm

$$\nabla^2(\sigma V) = 0 \quad (6)$$

Experimental tests were also performed to validate the finite element model. An acrylic tissue chamber was filled with three different concentrations of saline solution, 0.9%, 0.125%, and 0.02%. A linear motion stage was used to position the surface of the bioimpedance probe at discrete depths from 1 mm to 11 mm from the bottom of the tissue chamber. At each depth, bioimpedance readings were collected at ten frequencies exponentially distributed from 100 Hz to 100 kHz. Results from both the physical and FEA were then compared.

Fig. 4 Schematic of the FEA used to analyze potential distribution within a cylindrical column of saline. A normal current density is defined at the HC electrode and a ground condition was defined at the LC electrode (see Fig. 3). All other external boundaries were given an insulation boundary condition, and all internal boundaries were given a continuity boundary condition.

4.4 Soft Tissue Measurements to Validate BIS Instrument.

Cylindrical tissue samples were punched out of whole porcine spleen using a sharpened 25.4 mm diameter leather punch (McMaster-Carr, Cleveland, OH). The bioimpedance probe was positioned on top of the tissue and a 13-point logarithmic frequency sweep was performed. Voltage, current, and phase data were recorded by the PXI-5044 oscilloscope. The measured data were compensated for known magnitude and phase adjustments induced by the IA as well as the hook effect [35]. A nonlinear least squares method using a trust-region algorithm was used to fit the real part of the impedance data as shown in Eq. (7). Where $R(\omega)$ is the real part of the frequency-dependent impedance, R_{∞} is the resistance at infinite frequency, R_0 is the DC resistance, ω is the natural frequency, and τ is the time constant of the system. This method was previously proposed by Ayllon et al. [47] due to the Kramers–Kronig relation showing that the real and imaginary parts of a complex function, which is analytic in the upper half-plane of an impedance plot are coupled [48–50].

$$R(\omega) = R_{\infty} + \frac{(R_0 - R_{\infty}) \left(1 + (\omega\tau)^z \cos \frac{\alpha\pi}{2} \right)}{1 + 2(\omega\tau)^z \cos \frac{\alpha\pi}{2} + (\omega\tau)^{2z}} \quad (7)$$

5 Results and Discussion

This section will present the results of the experimental investigations performed. Due to the number of topics for which results are provided, a brief discussion will be included along with each result.

5.1 Output Impedance of the MHCP VCCS. The theoretical, simulated, and experimentally measured output impedances are shown in Fig. 5. Experimentally measured output impedances are nearly 1 M Ω through 50 kHz before dropping off to 25 k Ω at 1 MHz. The results compare favorably to both the simulated and theoretical output impedance at high frequencies while the theoretical output impedance is seen to be about 10 $\times$ that of the simulated and experimentally measured output impedance at low frequencies. The difference between the theoretical and simulated output impedances was investigated by determining the CMRR of the OP282 Spice model using the method described by Analog Devices [51]. At low frequency (1 kHz), the CMRR of the Spice model was found to be 61 dB, deviating from the datasheet, which states a minimum CMRR of 70 dB with a typical CMRR of 90 dB. It was decided that the model would be used as provided, since it was expected that impedance mismatches in the physical circuit would most likely degrade the CMRR in any event. The agreement between the experimentally measured and simulated results supported this hypothesis.

Fig. 5 Theoretical, simulated, and measured output impedance for the DC-stabilized MHCP

5.2 Performance of the MHCP VCCS. Since the output impedance is 45x larger than the worst case (highest) theoretical load impedance of 21 k Ω , shown in Fig. 2, and this ratio increases with increasing frequency, it was expected that the VCCS should perform favorably for the intended measurements.

To evaluate the DC compensation circuit, the DC offset voltage measurements from the simulations for each of the circuits were plotted as a function of load as shown in Fig. 6. The simulations revealed a linear load-dependent increase of the DC offset voltage for the standard MHCP design. Values range from 100 μ V at 100 Ω to 100 mV at 100 k Ω . This trend introduces a significant limitation to the performance of a VCCS. For example, if a ± 10 V supply was used for the amplifiers this would limit the maximum load impedance to only 10 k Ω . Assuming a VCCS output impedance of 1 M Ω , the DC current would be blocked by series output capacitance and have to flow through the output impedance to ground). Attempting to supply the requested current, the voltage at the amplifier output would saturate, removing or distorting any AC output signal intended for the load impedance. However, the DC-stabilized VCCS exhibits a load-independent DC offset voltage of approximately 600 μ V with a slight roll-off in DC offset voltage at higher loads down to 100 μ V at 100 k Ω . This significantly increases the range of load impedances the MHCP VCCS is able to drive.

The actual output current for the DC-stabilized VCCS circuit is shown in Fig. 7 for a 60 μ A RMS theoretical output current. The VCCS is capable of driving loads from 100 Ω to 1 k Ω through 200 kHz with less than a 1% error, and from 200 kHz through 1 MHz with less than 5% error. The 2 μ A decrease in current delivered to a 1 k Ω load at higher frequencies is due to the reduced ratio between the output impedance and the load impedance. In this situation, a larger part of the output current is diverted through the output impedance instead of through the load impedance.

The metrics discussed in this section indicate that the MHCP VCCS provides suitable performance for the intended measurements and also provides improved performance over the standard MHCP design.

5.3 Stray Capacitance Compensation. A typical plot of susceptance versus frequency is shown in Fig. 8. At frequencies over 100 kHz, the susceptance is seen to increase linearly. As proposed by Buendia et al. [35], susceptance at high frequencies is due to parasitic capacitance entirely, and thus the slope of this graph at high frequencies is equal to the parasitic capacitance present in the experimental setup. Stray capacitance measurements for 15 porcine spleen samples were made, with the average average stray capacitance was determined to be 712 ± 182 pF and was compensated for by the method described.

Fig. 6 Comparison of DC offset voltage measured in MultiSim for a standard and DC stabilized VCCS as a function of load

Fig. 7 Measured RMS current output for low and high impedance loads for the DC-stabilized MHCP

5.4 Influence of Measurement Volume Geometry. The FEA determined voltage drop between the two middle electrodes at a 11 mm depth in a 0.9% saline solution was 2.2 mV while the voltage drop in 1 mm of solution was 9.7 mV, resulting in an impedance of 37 Ω at 11 mm and 162 Ω at 1 mm. The measured impedance at 1 mm was therefore 4.4x greater than that at 11 mm. The solid line in Fig. 9(a) shows the results at each probe height from 1 mm to 11 mm and shows an exponential increase in measured voltage, and therefore impedance, as the probe gets closer to the bottom of the container. Similar results were obtained for each simulated conductivity.

The results obtained from experimental measurements on saline solutions showed excellent agreement with the FEA results, exhibiting values of 2.3 ± 0.04 mV and 9.6 ± 0.2 mV, resulting in an impedance of 38 Ω at 11 mm and 160 Ω at 1 mm. The experimental impedance at 1 mm was therefore 4.2x greater than that at 11 mm. Minimal variation in output current was seen.

Even though there is usually little that can be done to eliminate this effect, it is important to be aware that it exists and may influence measurements. In certain situations it may be possible to minimize or compensate for this effect, but this is beyond the scope of this article

5.5 Bioimpedance of Porcine Spleen Tissue. After the system performance was evaluated and as a final test of its performance, 15 porcine spleen tissue samples were measured with the BIS instrument. All spleen samples were uncompressed with lengths >10 mm, with diameters equivalent to the probe diameter. The data were fit to frequency measurements up to 100 kHz. In

Fig. 8 Susceptance plot showing a linear increase as frequency is increased. The slope of this line is used to compensate for the parasitic capacitance in the system.

Fig. 9 Results from the numerical and experimental effect of probe proximity to the bottom of the experimental tissue chamber, h . (a) Top view of the potential distribution (shading) and current density (streamlines) for $h = 11$ mm at the probe surface (left) and for $h = 1$ mm at the probe surface (right). Potential drop between the middle two electrodes is seen to increase in the case on the right. (b) Plot of impedance normalized to the measured impedance at 11 mm ($|Z|/|Z^*|$) versus h . For the finite element model, impedance was calculated by dividing the potential drop by 60 μ A. For the experimental measurements, impedance was calculated by dividing the measured potential drop by the measured current. The plot shows a dramatic increase in measured impedance as the probe moves closer to the bottom of the acrylic tissue chamber.

Fig. 10, the raw data are shown (Z_{raw}) along with the adjusted data after compensating for the height of the tissue sample and the Hook effect (Z_{cor}) as discussed above. The solid curve (Z_{fit}) shows the nonlinear least squares fitted result for the Cole model. The fit equation constrains the curve to a semicircular shape, however, the alpha term allows vertical movement along the y-axis. The fit is defined mainly from the preceding eight data points, since the last three data points are not able to overcome the bias of the other data points.

In Fig. 11, the calculated average permittivity and conductivity values for the experimental samples are plotted along with values

Fig. 10 Raw (Z_{raw}), corrected (Z_{cor}), and fitted data (Z_{fit}) for an example bioimpedance measurement on porcine spleen tissue. The order of points from left to right in the figure each represents an individual measurement performed at frequencies of 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, and 50.0 kHz, respectively.

Fig. 11 Comparison of electrical conductivity (σ) and electrical permittivity (ϵ) experimental results for porcine spleen with the literature (note literature based on a range of animal spleen values at various temperatures). Errors bars are provided, but are not clearly legible due to the log axis scale. The measurement error for conductivity values were fairly consistent, ranging from about $\pm 21\%$ at 0.1 kHz down to $\pm 18\%$ at 100 kHz. Permittivity measurement errors below 20 kHz were $\pm 36\%$, decreasing to about $\pm 20\%$ over the 1–20 kHz range, and again increasing to $\pm 36\%$ by 0.5 kHz, and $\pm 49\%$ by 0.2 kHz.

from the literature [17]. The measured conductivity values, ϵ_{exp} , are slightly low compared to the literature values, ϵ_{lit} , while the permittivity values, σ_{exp} and σ_{lit} , were found to be slightly high. However, much of the cited literature values were taken at body temperature, while the ex vivo experiments performed here were done at room temperature. Compensating for the 2%/°C increase in electrical conductivity values puts the measurements performed in this research within the range of the reported literature. These results illustrate that the designed circuit and compensation measures were able to accurately quantify the tissue measured.

6 Conclusions

In this research, a laboratory BIS instrument for tissue characterization was designed and evaluated, with compensation techniques implemented for several common issues seen when making bioimpedance measurements.

A novel constant current source was implemented to actively minimize DC offset voltage and optimize the dynamic range of the system. The DC offset voltage was shown to be load-independent and remain lower than 625 μ V over a broad range of loads while still maintaining an output impedance of 950 k Ω through 100 kHz. A similar current source without the DC-stabilizing circuitry showed heavy load-dependent DC offset voltages.

In addition, the effect of discontinuities in the electrical properties of the measurement volume was shown to increase impedance measurements by as much as 4x. This was demonstrated by moving the bioimpedance probe to within 1 mm of the bottom of an acrylic measurement container filled with a saline solution. While not a major concern in this research, other areas where tissue thickness changes dramatically, such as in monitoring bioimpedance during electrosurgery and traumatic compressive blast events, will need to account for this effect.

Parasitic capacitance was shown to be an important factor to account for and was seen to dominate the bioimpedance readings at frequencies greater than 100 kHz. For the BIS instrument, it was measured to be 712 pF on average for the circuit. Within the experimental setup, this effect is believed to limit the use of the proposed current source circuit for frequencies higher than 100 kHz.

An additional limitation was that the hook effect was seen in the measurements. Since the hook effect is typically a result of parasitic capacitance, we are confident that future design revisions will

not only minimize this effect, but also move it to a higher frequency range that is outside the range of interest. Typically commercial BIS systems have been reported to not show this effect until much higher frequencies. Future work will therefore focus on minimizing the hook effect as well as performing comparative measurements with existing instrumentation as a benchmark. In addition, the compensation for tissue thickness and parasitic capacitance will be performed in software to realize real-time dynamic bioimpedance measurements of tissue. Recent work shows the importance of accounting for these conditions in studying the effect of bioimpedance of soft tissue under compression [52].

Finally, the electrical resistivity and permittivity values for spleen tissue were measured using the BIS system and shown to compare favorably with previously published values, thus providing a final successful validation of the BIS instrument.

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