

1 Electrical impedance characterization of normal
2 and cancerous human hepatic tissue

3 Shlomi Laufer¹, Antoni Ivorra², Victor E. Reuter³, Boris Rubinsky^{1,2*}, Stephen B.
4 Solomon⁴

5 ¹ Research Center for Bioengineering in the Service of Humanity and Society,
6 School of Computer Science and Engineering, Hebrew University of Jerusalem, Israel

7 ² Depts. of Bioengineering and Mechanical Eng., University of California at Berkeley, California, USA

8 ³ Department of Pathology Memorial Sloan-Kettering Cancer Center, New York, New York

9 ⁴ Department of Radiology Memorial Sloan-Kettering Cancer Center, New York, New York

10 * To whom the correspondence should be addressed at <rubinsky@cs.huji.ac.il>

11
12
13

14 Abstract

15 The four-electrode method was used to measure the ex-vivo complex electrical
16 impedance of tissues from 14 hepatic tumors and the surrounding normal liver from 6
17 patients. Measurements were done in the frequency range of 1 kHz - 400 kHz. It was
18 found that the conductivity of the tumor tissue was much higher than that of the normal
19 liver tissue in this frequency range (from $0.14 \pm 0.06 \text{ S m}^{-1}$ vs. $0.03 \pm 0.01 \text{ S m}^{-1}$ at 1
20 kHz to $0.25 \pm 0.06 \text{ S m}^{-1}$ vs. $0.15 \pm 0.03 \text{ S m}^{-1}$ at 400 kHz). Cole-Cole models were
21 estimated from the experimental data and the four parameters ($\rho_0, \rho_\infty, \alpha, f_c$) were
22 obtained using a least squares fit algorithm. The Cole-Cole parameters for cancerous and
23 normal liver are ($9 \pm 4 \ \Omega \text{ m}^{-1}, 2.2 \pm 0.7 \ \Omega \text{ m}^{-1}, 0.5 \pm 0.2, 140 \pm 103 \text{ kHz}$) and
24 ($50 \pm 28 \ \Omega \text{ m}^{-1}, 3.2 \pm 0.6 \ \Omega \text{ m}^{-1}, 0.64 \pm 0.04, 10 \pm 7 \text{ kHz}$), respectively. These data
25 can contribute to developing bioelectric applications for tissue diagnostics, and in tissue
26 treatment planning with electrical fields such as radiofrequency tissue ablation,
27 electrochemotherapy and gene therapy with reversible electroporation, nanoscale pulsing
28 and irreversible electroporation.

29
30

32 Introduction

33 This study reports new experimental data on the complex electric properties of normal
34 and malignant human liver tissue. Measurement of the electric impedance properties of
35 tissue is not new and the fact that diseased and healthy tissues have different properties is
36 well known and used in medical applications. However, the field of bioengineering in
37 general and bioelectric engineering in particular has an abundance of theoretical or
38 technology papers, but lacks tissue property data – in particular human tissue data.
39 Therefore, it is our belief that any additional measured tissue data is a welcome
40 contribution to the field. Measurements from human tissue are much more difficult to
41 obtain and are more relevant to engineering applications. Several large scale studies have
42 been published on electric impedance properties of healthy tissue; perhaps the largest one
43 is that published by Gabriel in 1996 (Gabriel *et al.*, 1996a; Gabriel *et al.*, 1996b, c). That
44 study provides data on a large number of various healthy tissues but does not deal with
45 malignant tissues. Furthermore, the lower frequency range (below 1MHz) was not
46 covered in depth. Gabriel's recent work (Gabriel *et al.*, 2009) deals with this lower
47 frequency range but once again only for normal tissue . When dealing with cancerous
48 tissue, for which many practical applications of bioelectric engineering exist, this crucial
49 basic data are very sparse or missing. For example, hundreds of papers have been
50 published on EIT (electrical impedance tomography) of the breast. However, to the best
51 of our knowledge there are only two major studies which report data on the electric
52 properties of human breast cancer tissue (Surowiec *et al.*, 1988; Jossinet and Schmitt,
53 1999). More comprehensive data on cancer tissue properties would have obvious benefits
54 as regards existing applications. Acquiring such data for the prostate and the breast is
55 currently the focus of the Dartmouth group (Halter *et al.*, 2009b, a; Halter *et al.*, 2009c).
56 With regards to the liver there are several new treatments for cancer which require
57 knowledge of the electric properties of liver cancer tissue such as the treatment of cancer
58 by radiofrequency (RF) induced thermal ablation. In this minimally invasive form of
59 treatment, RF currents (around 400-500 KHz) are injected into the tumor to elevate the
60 temperature through a Joule heating effect to levels that ablate it (Curley *et al.*, 1997;

61 Goldberg, 2001). Many computer models have been developed to simulate and explore
62 the behavior of electric fields in tumors and normal tissues (Haemmerich *et al.*, 2003b;
63 Liu *et al.*, 2005 ; Chang and Nguyen, 2004; Berjano, 2006). These models show that the
64 ratio of healthy to malignant tissue electric conductivity has an important influence on the
65 outcome of the treatment (Liu *et al.*, 2006; Solazzo *et al.*, 2005). These studies are
66 crucial for understanding and planning RF treatment, but it is all the more concerning to
67 see that the data reported are usually only of healthy tissue. A recent study (Dieter and
68 et al., 2009) is one of the only ones to date to address the issue of the lack of data on
69 malignant tissue , and in this context our independent study has value as adding to the
70 findings of their work. We would like to mention that the general knowledge that electric
71 properties of cancerous tissue differ from healthy tissue properties is not enough. For
72 example, in the prostate, the malignant tissue has lower electric conductivity than the
73 healthy tissue (Halter *et al.*, 2009a) whereas in the liver the malignant tissue has higher
74 conductivity.

75 Our group has two particular reasons for researching the electric properties of cancerous
76 human liver tissue. The first is a new emerging minimally invasive tissue ablation
77 technique called Non Thermal Irreversible Electroporation (NTIRE). In irreversible
78 electroporation, micro- to millisecond high field electric pulses are delivered to
79 undesirable tissue to produce cell necrosis by inducing nanoscale defects in the cell
80 membrane lipid bilayer. In the non-thermal mode the pulses are designed in such a way
81 as to avoid substantial Joule heating- induced tissue damage due to temperature elevation.
82 Hence NTIRE is a molecular surgery procedure, in the sense that it affects only one
83 molecular component of the tissue – the cell membrane lipid bilayer. Details on the field
84 can be found in a new book (Rubinsky, 2010)

85 Second, we have also developed a classifier- based diagnostic tool based on bio-
86 impedance data. Elsewhere we have shown how bio-impedance can be used to classify
87 tissue types. Although using electric tissue properties for diagnostics is not new, we
88 believe that the classifier- based methods we develop are. It is important to recognize that
89 in developing classifiers a large database of experimental measurements is absolutely
90 essential. Our previous studies on designing classifiers for tissue diagnostics were

91 performed using computer simulations (Laufer and Rubinsky, 2009b; Laufer *et al.*, 2009)
92 or animal models data (with no cancer) (Laufer and Rubinsky, 2009a). We used these
93 partial substitutes for actual human tissue data, because no relevant human data are
94 available in the literature. In the current study we provide the data needed for developing
95 classifiers. To the best of our knowledge this work is the first to publish the permittivity
96 (and not just conductivity) of liver cancer in the reported frequency range. While
97 conductivity is the main property needed for RF and IRE simulations, the complex phase
98 response can be critical when classifying tissue. In our opinion substantially more studies
99 on human tissue data are needed to further develop the use of classifier techniques in
100 diagnostics.

101

102

103 Materials and methods

104 EIS measurement

105 The measurements were performed using the four electrode method. The electrodes were
106 made from 26 gauge (0.463 mm) hypodermic stainless steel needles. The needles were
107 scratched using sandpaper to maximize their effective area and thus minimize their
108 interface impedance (Geddes, 1972) . Two types of configurations were made and can be
109 seen in Figure 1. In the first configuration the needles were placed in a straight line with a
110 2.3 mm distance between needles and a 5.5 mm exposure. The two outer needles were
111 used for injecting current while the two inner needles were used for measuring the
112 voltage. In the second configuration the needles were placed in a square shape with a 2.3
113 mm distance and a 5.5 mm exposure. The two top needles were used for current injection
114 while the two bottom needles were used for voltage measurement. Although the first
115 configuration gives a better SNR, since the measurement electrodes are in the line of the
116 current electrodes, the second configuration was employed for smaller tumors because of
117 its compact size.

118 All impedance measurements were performed using a custom-made impedance analyzer
119 embedded in a single Printed Circuit Board (PCB). The impedance analyzer architecture
120 is described in (Ivorra and Rubinsky, 2007). A total of 11 logarithmically spaced
121 frequencies, ranging from 1 kHz to 400 kHz, were measured. Each scan of all 11
122 frequencies took 30 ms and each measurement was performed for at least 5 seconds. The
123 maximum applied current was 100 μA . The calculated current density calculated from
124 the surface area of the electrode is 1.25 mA/cm^2 and the RMS value is 0.88 mA/cm^2 .

125

126

127

128 Bioimpedance relationships and measurement error evaluation

129 Bioimpedance is a diagnostic method based on the study of the passive electrical
130 properties of biological tissues. When applying an alternating voltage, V , on a tissue the
131 impedance, Z , is given by the ratio between this voltage and the resulting current, I ;
132 namely $V = I \cdot Z$. Alternatively we can use the admittance value, Y , which is given by
133 $Y = 1/Z$. Typically admittance combines conductive (G) and capacitive (C) components
134 such that $Y = G + j\omega C$, where $\omega = 2\pi f$ and j is the complex number $\sqrt{-1}$. G and C
135 depend on the properties of the tissue and on the geometry of the measurement. They are
136 therefore typically separated into two parts:

$$G = K \cdot \sigma \quad \text{and} \quad C = K \cdot \epsilon_0 \epsilon_r \quad (1)$$

137

138 where σ is the conductivity of the tissue (expressed in S/m), ϵ_r is the relative permittivity
139 of the tissue, ϵ_0 is the permittivity of vacuum ($8.85 \times 10^{-12} \text{F}/\text{m}$) and K is the cell
140 constant, i.e., the geometric scaling factor of the measurement: cell = area/length
141 (expressed in $\text{m}^2/\text{m} = \text{m}$). Since G and C are the measured parameters and σ and ϵ_r
142 are the parameters that characterize the tissue, we first need to evaluate K .

143 In a recent study by Gabriel (Gabriel et al., 2009), the authors suggest a method to
144 measure the cell constant and evaluate the noise of the measurement. We implemented
145 this method and performed new measurements. The conductance of five NaCl solutions
146 ranging from 0.001 to 0.15 M was measured (this range was chosen since it covers the
147 range of values for the different tissues we studied). Theoretically ionic solutions such as
148 aqueous NaCl exhibit no dielectric dispersion at frequencies below 1 MHz and the ratio
149 between the conductivity of these different solutions is known. This means that
150 comparing the different cell constants for different frequencies and different solutions can
151 be used to estimate the total effect of equipment noise and that of different experimental
152 artifacts such as electrode polarization. In this study we performed such an analysis. The
153 measurements can be seen in Figure 2 and the results are summarized in Tables 1-3. The
154 method used was the same as that suggested by Gabriel et al. and a complete description
155 can be found there. The only difference was that we also evaluated the phase data since
156 phase was measured as well. It can be seen that for solutions 0.005 to 0.15 M the error
157 was below 9% (compared to 10% in Gabriel et al.). For 0.001 M a higher error of 23%
158 was present (compared to 67% in Gabriel et al.), but this solution has very low
159 conductivity, much lower than that of body tissue, and therefore is not relevant to this
160 study. It can also be seen that for the second needle configuration and a concentration of
161 0.15M the standard deviation is relatively high (12%). This is due to the fact that the total
162 impedance is less than 10 ohm which is around the limits of our device. Since the liver
163 has higher resistive values, this does not influence this experiment either. The phase of
164 NaCl solutions at these frequencies should be zero and therefore any measured phase can
165 be regarded as an error or artifact (e.g. electrode polarization). In general the phase was
166 around 2^0 with the exception of low NaCl concentration (0.001M) in the higher
167 frequency range (100 kHz ~ 400 kHz). As mentioned the tissue measured had higher
168 conductivities and consequently this did not influence our measurements.

169

170 Tissue handling

171 This study was approved by the Institutional Review Board at the Memorial Sloan
 172 Kettering Cancer Center. All the tissue handling was performed in the Pathology
 173 Department under the supervision of one of the hospital pathologists. Measurements were
 174 made within 1-2 hours of tissue resection. Livers from six patients (3 female and 3 male,
 175 4 with colon metastases and 2 with primary liver cancer) with 14 different tumors were
 176 examined. Patients were 42 to 81 years old (average age 58), and tumor size was
 177 between 0.8 cm to 5 cm (average 2 cm). Of the six livers one had cirrhosis. Due to the
 178 large size of the livers (around 10 cm) and some of the tumors, multiple measurement
 179 points were used. A total of 26 points of healthy liver, 32 points of cancerous liver and 7
 180 points of cirrhotic liver were measured. An example of the measurement configuration
 181 can be seen in Figure 3.

182

183 Cole-Cole model fitting

184 For the parameterization of the frequency-dependent impedance, the Cole-Cole
 185 dispersion model (Kenneth and Robert, 1941; Jossinet and Schmitt, 1999; Halter *et al.*,
 186 2008) was used, with

$$\rho(f) = \rho_{\infty} + \frac{\rho_0 - \rho_{\infty}}{1 + (jf/f_c)^{\alpha}} \quad (2)$$

187 where $\rho(f)$ is the complex resistivity given by $\rho = 1/\sigma^*$ where $\sigma^* = \sigma + j\omega\epsilon_0\epsilon_r$, ρ_{∞} is
 188 the high frequency resistivity, ρ_0 is the low frequency resistivity, f_c is the characteristic
 189 frequency, α is the fractional power representing the depression of the circular arc from
 190 the x-axis.

191 The four parameters ($\rho_0, \rho_{\infty}, f_c, \alpha$) were estimated with MATLAB's function `fsolve` using
 192 the Levenberg-Marquardt method. As in (Halter *et al.*, 2008) the quality of the estimation
 193 was evaluated using the goodness criterion:

$$\epsilon = \frac{1}{N} \sum_{i=1}^N |\rho_m(f_i) - \rho_e(f_i)| \quad (3)$$

194 where $\rho_m(f_i)$ and $\rho_e(f_i)$ are the measured and estimated impedances at each frequency,
195 respectively, and N is the number of frequencies (in our case 11).

196 The initial parameters were set in the following manner (Halter *et al.*, 2008):

197 ρ_∞ : Real part of impedance at the maximum frequency, 400 kHz.

198 ρ_0 : Real part of impedance at the minimum frequency, 1 kHz.

199 f_c : Frequency at which $Im(\rho_m(f_i)) > Im(\rho_m(f_{i-1}))$ & $Im(\rho_m(f_i)) > Im(\rho_m(f_{i+1}))$
200 first occurs as the frequency proceeds from low to high $i = 2, 3, \dots, N - 1$.

201 α : Set to 1 for all cases.

202

203 Results

204

205 When presenting bioimpedance data using the matching Cole-Cole models two
206 approaches can be taken. In the first approach the impedance values from all the tissue
207 data sets are initially averaged and then these average values are used to extract the Cole-
208 Cole parameters. In the second approach a Cole-Cole model is developed for each tissue
209 data set and the model parameters are averaged over all the data sets. Due to variations
210 between tissues these two methods will yield different results. Furthermore, while the
211 first method gives the standard deviation of the impedance values, in the second method
212 the standard deviation will be of the Cole-Cole parameters. Averaging the impedance
213 values has the advantage of averaging the data that was actually measured and avoiding
214 the model fitting stage, which is known to introduce errors. When examining the
215 correlation between the different impedance values for different frequencies, we found
216 them to be much higher (~ 0.9) than those of different Cole-Cole parameters (~ 0.45).
217 This would suggest that when adding independent and identically distributed (i.i.d) noise
218 to simulated data (for example in computer models) it would be better to use the Cole-
219 Cole model and add the noise to Cole-Cole parameters and not to the impedance values.
220 Given these considerations we decided to present the Cole-Cole parameters obtained
221 from both methods. The average and standard deviation of the electrical conductivity and
222 the relative permittivity can be found in Table 4 and are plotted in Figure 4. As a

223 reference the data for normal liver from (Gabriel *et al.*, 1996b) are also presented in
224 Figure 4. The average impedance values can be seen in Figure 5 and the average
225 admittance in Figure 6. The Cole-Cole parameters were also calculated for each of the
226 measurements. The average error between the model and the measured data was $0.1 \pm$
227 0.1Ω (approximately 1%). A sample of these models can be seen in Figure 7, and the
228 average and standard deviation are presented in Table 5.

229

230 Discussion

231 Previous studies of the liver, both animal and human, (Smith *et al.*, 1986; Joines *et al.*,
232 1994; Haemmerich *et al.*, 2003a; Stauffer *et al.*, 2003; O'Rourke *et al.*, 2007; Dieter and
233 et al., 2009) showed that cancerous tissue has higher conductivity values than healthy
234 tissue. In our study we found the same relationship. These ratios are not trivial since in
235 prostate cancer the tumor has lower conductivity than the surrounding healthy tissue
236 (Halter *et al.*, 2008; Halter *et al.*, 2009b) while in breast cancer the tumor has higher
237 conductivity than the surrounding fat typically found at older ages, but lower than
238 glandular tissue (Jossinet and Schmitt, 1999).

239 It has also been found that the difference in electrical conductivity values is much higher
240 in the lower frequency range, as reported in (Dieter and et al., 2009). In (Haemmerich
241 and Wood, 2006; Dieter and et al., 2009) it was suggested that the RF ablation frequency
242 (currently around 400-500 kHz) should be reduced. It was shown that although the
243 conductivity of malignant tissue is higher at all frequencies, the difference is much higher
244 at lower frequencies and therefore performing the ablation process at lower frequencies
245 will cause the current to preferentially heat the malignant tissue. This will cause better
246 destruction of the tumor with less damage to surrounding tissue. Further studies should
247 examine impedance values on the border of the tumor. If they are closer to those of
248 healthy tissue, then reducing the frequency might accelerate the necrosis of the center of
249 the tumor, where lower conductivities exist, while leaving the boundary alive to
250 regenerate. Another issue is that the difference between ablated tissue and non ablated
251 tissue is much higher at the lower frequency range (Dieter and et al., 2009). Once again
252 this can encourage the electrical currents and the resulting heating to concentrate in the
253 ablated area where the electrical conductivity is higher. Although using higher

254 frequencies seems less efficient, the heating process might be more homogenous and thus
255 more predictable. These parameters should be used in future computer simulations, and
256 more ex-vivo RF ablation experiments should be performed for a better understanding of
257 the problem.

258 In addition, it was seen that the β dispersion central frequency of the malignant tissue was
259 much higher than that of the normal tissue (155.7 kHz and 10.5 kHz respectively). The β
260 dispersion depends on the cell membrane capacity. Since regions of cell necrosis are
261 commonly found inside tumor tissue, the capacitive effect of the intact cell membrane in
262 those areas is reduced. As mentioned the conductivity at the lower frequency range is
263 much higher in cancerous tissue. This could be due to higher extra-cellular water content
264 in the tumor and lower cell membrane density due to necrosis. At the high frequency
265 range the conductivity is still higher, although less significant than in the lower range,
266 this would suggest intracellular changes as well.

267 Only one liver was cirrhotic and thus further studies need to be done. The conductivity of
268 this liver was much higher than of the healthy liver and it had a reduced phase response
269 in comparison to healthy tissue. The considerable difference between healthy and
270 cirrhotic liver could suggest bio-impedance as a method for non-invasive liver evaluation
271 (using methods similar to Electrical Impedance Tomography).

272 Another interesting fact was that while for all the healthy tissues a Cole-Cole model was
273 easily fit, the frequency response for some of the cancerous tissue was “flat” and thus the
274 model appeared not to fit. This phenomenon has been reported in previous studies
275 (Antoni and et al., 2009; Jossinet and Schmitt, 1999) and an example can be seen in
276 Figure 7. This could influence the relatively large variability of parameter f_c in the Cole-
277 Cole model.

278 The wide variability of the data in this study is a disturbing matter. We found the
279 variability to be around 25% for healthy tissue and 40% for cancerous tissue, while the
280 consistency of our equipment was better than 9% and on average around 5%. However,
281 similarly large variability is not unusual and has been reported in other studies as well
282 (Halter *et al.*, 2009a; Jossinet and Schmitt, 1999). A possible explanation can be found in
283 Haemmerich *et al.*, 2002 which shows that the conductivity in an animal model decreases
284 by 53% at 10 Hz and by 32% at 1 MHz during the first two hours after liver removal.

285 Since in a clinical setup we cannot control the exact time between removal of the liver
286 and the time when the tissue properties are measured, this can influence the variability of
287 the data, as was pointed out by (Halter *et al.*, 2009a). The effect of the time lag on
288 measurement observed by Haemmerich *et al.* coincides to some extent with the fact that
289 in our data the variability was higher at the lower frequency range. This variability in the
290 lower frequency range, especially in the permittivity, could also be due to electrode
291 polarization which is more dominant in this frequency range. In four of the livers we
292 measured more than four areas and we calculated the standard deviation. We found it to
293 be 15% for healthy tissue and 25% for cancerous tissue. Since these measurements were
294 performed approximately at the same time this suggests that these values represent the
295 measurement accuracy and tissue inhomogeneity whereas the remainder can be ascribed
296 to patient variability and the time lag between tissue extraction and measurement. The
297 larger variability in tumors is probably due to their irregular form. It was also shown in
298 (Haemmerich *et al.*, 2002) that after 24 hours the conductivity values rise again. This
299 could explain why on average we obtained lower values than those found by (Gabriel *et*
300 *al.*, 1996b). The variability in the data further emphasizes the need for many more
301 experimental studies on physiological measurements of human tissue data for
302 bioimpedance applications. Further studies should also examine whether the time of
303 ischemia affects differently malignant and normal liver tissue.

304

305

306 Conclusion

307 The electrical conductivity and permittivity of ex-vivo malignant and healthy liver tissue
308 was measured. It was found that malignant tissue had higher conductivity values and a
309 lower phase response in the entire measured frequency range (1 kHz-400 kHz). These
310 data can be used for both bio-impedance needle guidance and to improve the RF ablation
311 procedure. In this work we did not distinguish between different types of liver cancers
312 due to the small amount of data and IRB constraints. This should be examined in larger
313 samples and the feasibility of using bio-impedance measurements as a classifier should
314 be evaluated. Furthermore it would be interesting to compare malignant tumors to benign
315 tumors; these data could be used to construct bio-impedance based tissue classifiers

316 (Laufer and Rubinsky, 2009a, b). Examining the homogeneity of impedance values over
317 the entire tumor, including its borders, could help improve the RF ablation procedure.

318 References:

319

320 Andreuccetti D, Fossi R and Petrucci C 1997 Institute for Applied Physics "Nello Carrara" of the
321 Italian National Research Council. Calculation of the dielectric properties of body tissues
322 in the frequency range 10 Hz - 100 GHz. Florence (Italy)

323 <http://niremf.ifac.cnr.it/tissprop/>

324 Antoni I and et al. 2009 In vivo electrical conductivity measurements during and after tumor
325 electroporation: conductivity changes reflect the treatment outcome *Physics in*
326 *Medicine and Biology* **54** 5949

327 Berjano E J 2006 Theoretical modeling for radiofrequency ablation: state-of-the-art and
328 challenges for the future *Biomed Eng Online* **5** 24

329 Chang I A and Nguyen U D 2004 Thermal modeling of lesion growth with radiofrequency
330 ablation devices *Biomed Eng Online* **3** 27

331 Curley S A, Davidson B S, Fleming R Y, Izzo F, Stephens L C, Tinkey P and Cromeens D 1997
332 Laparoscopically guided bipolar radiofrequency ablation of areas of porcine liver *Surg*
333 *Endosc* **11** 729-33

334 Dieter H and et al. 2009 Electrical conductivity measurement of excised human metastatic liver
335 tumours before and after thermal ablation *Physiological Measurement* **30** 459

336 Gabriel C, Gabriel S and Corthout E 1996a The dielectric properties of biological tissues: I.
337 Literature survey *Phys Med Biol* **41** 2231-49

338 Gabriel C, Peyman A and Grant E H 2009 Electrical conductivity of tissue at frequencies below 1
339 MHz *Phys Med Biol* **54** 4863-78

340 Gabriel S, Lau R W and Gabriel C 1996b The dielectric properties of biological tissues: II.
341 Measurements in the frequency range 10 Hz to 20 GHz *Phys Med Biol* **41** 2251-69

342 Gabriel S, Lau R W and Gabriel C 1996c The dielectric properties of biological tissues: III.
343 Parametric models for the dielectric spectrum of tissues *Phys Med Biol* **41** 2271-93

344 Geddes L A 1972 *Electrodes and the measurement of bioelectric events* Wiley-Interscience: New
345 York

346 Goldberg S N 2001 Radiofrequency tumor ablation: principles and techniques *Eur J Ultrasound*
347 **13** 129-47

348 Haemmerich D, Ozkan R, Tungjitkusolmun S, Tsai J Z, Mahvi D M, Staelin S T and Webster J G
349 2002 Changes in electrical resistivity of swine liver after occlusion and postmortem *Med*
350 *Biol Eng Comput* **40** 29-33

351 Haemmerich D, Staelin S T, Tsai J Z, Tungjitkusolmun S, Mahvi D M and Webster J G 2003a In
352 vivo electrical conductivity of hepatic tumours *Physiol Meas* **24** 251-60

353 Haemmerich D and Wood B J 2006 Hepatic radiofrequency ablation at low frequencies
354 preferentially heats tumour tissue *Int J Hyperthermia* **22** 563-74

355 Haemmerich D, Wright A W, Mahvi D M, Lee F T, Jr. and Webster J G 2003b Hepatic bipolar
356 radiofrequency ablation creates coagulation zones close to blood vessels: a finite
357 element study *Med Biol Eng Comput* **41** 317-23

358 Halter R J, Hartov A, Paulsen K D, Schned A and Heaney J 2008 Genetic and least squares
359 algorithms for estimating spectral EIS parameters of prostatic tissues *Physiol Meas* **29**
360 S111-23

361 Halter R J, Schned A, Heaney J, Hartov A and Paulsen K D 2009a Electrical properties of prostatic
362 tissues: I. Single frequency admittivity properties *J Urol* **182** 1600-7

363 Halter R J, Schned A, Heaney J, Hartov A and Paulsen K D 2009b Electrical properties of prostatic
364 tissues: II. Spectral admittivity properties *J Urol* **182** 1608-13

365 Halter R J, Zhou T, Meaney P M, Hartov A, Barth R J, Jr., Rosenkranz K M, Wells W A, Kogel C A,
366 Borsic A, Rizzo E J and Paulsen K D 2009c The correlation of in vivo and ex vivo tissue
367 dielectric properties to validate electromagnetic breast imaging: initial clinical
368 experience *Physiol Meas* **30** S121-36

369 Ivorra A and Rubinsky B 2007 In vivo electrical impedance measurements during and after
370 electroporation of rat liver *Bioelectrochemistry* **70** 287-95

371 Joines W T, Zhang Y, Li C and Jirtle R L 1994 The measured electrical properties of normal and
372 malignant human tissues from 50 to 900 MHz *Med Phys* **21** 547-50

373 Jossinet J and Schmitt M 1999 A review of parameters for the bioelectrical characterization of
374 breast tissue *Ann N Y Acad Sci* **873** 30-41

375 Kenneth S C and Robert H C 1941 Dispersion and Absorption in Dielectrics I. Alternating Current
376 Characteristics *The Journal of Chemical Physics* **9** 341-51

377 Laufer S and Rubinsky B 2009a Cellular phone enabled non-invasive tissue classifier *PLoS ONE* **4**
378 e5178

379 Laufer S and Rubinsky B 2009b Tissue Characterization With an Electrical Spectroscopy SVM
380 Classifier *Biomedical Engineering, IEEE Transactions on* **56** 525-8

381 Laufer S, Solomon S B and Rubinsky B 2009 A new linear algebra based mathematical technique
382 for Electrical Impedance Spectroscopy guided biopsy *Accepted to the 11th World*
383 *Congress on Medical Physics and Biomedical Engineering*

384 Liu Z, Ahmed M, Weinstein Y, Yi M, Mahajan R L and Goldberg S N 2006 Characterization of the
385 RF ablation-induced 'oven effect': the importance of background tissue thermal
386 conductivity on tissue heating *Int J Hyperthermia* **22** 327-42

387 Liu Z, Lobo S M, Humphries S, Horkan C, Solazzo S A, Hines-Peralta A U, Lenkinski R E and
388 Goldberg S N 2005 Radiofrequency tumor ablation: insight into improved efficacy using
389 computer modeling *AJR Am J Roentgenol* **184** 1347-52

390 O'Rourke A P, Lazebnik M, Bertram J M, Converse M C, Hagness S C, Webster J G and Mahvi D M
391 2007 Dielectric properties of human normal, malignant and cirrhotic liver tissue: in vivo
392 and ex vivo measurements from 0.5 to 20 GHz using a precision open-ended coaxial
393 probe *Phys Med Biol* **52** 4707-19

394 Rubinsky B 2010 Irreversible Electroporation In *Series in Biomedical Engineering* Springer pp
395 X1V, 314

396 Smith S R, Foster K R and Wolf G L 1986 Dielectric properties of VX-2 carcinoma versus normal
397 liver tissue *IEEE Trans Biomed Eng* **33** 522-4

398 Solazzo S A, Liu Z, Lobo S M, Ahmed M, Hines-Peralta A U, Lenkinski R E and Goldberg S N 2005
399 Radiofrequency ablation: importance of background tissue electrical conductivity--an
400 agar phantom and computer modeling study *Radiology* **236** 495-502

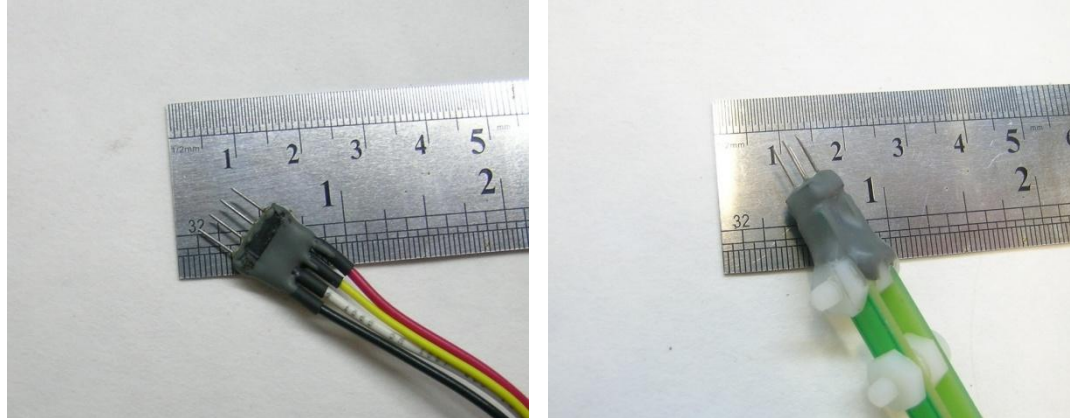
401 Stauffer P R, Rossetto F, Prakash M, Neuman D G and Lee T 2003 Phantom and animal tissues
402 for modelling the electrical properties of human liver *Int J Hyperthermia* **19** 89-101

403 Surowiec A J, Stuchly S S, Barr J R and Swarup A A S A 1988 Dielectric properties of breast
404 carcinoma and the surrounding tissues *Biomedical Engineering, IEEE Transactions on* **35**
405 257-63

406

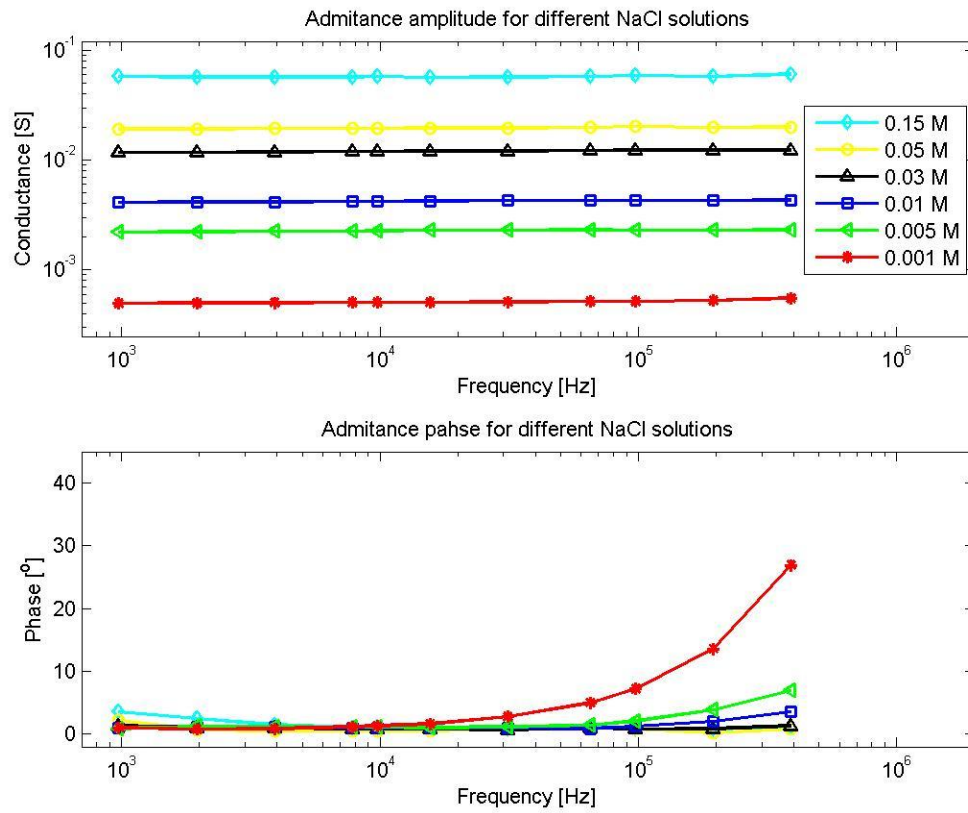
407

408



409 Figure 1: The four electrode devices custom-made for the experiment. (a) The needles are
410 set in a straight line. (b) The needles are set in a square.

411



412

413 Figure 2: Admittance of different NaCl solutions measured using electrode (a) for cell
 414 constant calculation.

415

416

NaCl [M]	G [S] average	G [S] SD	G [S] %SD	$\sigma[S m^{-1}]$ average	$\sigma[S m^{-1}]$ Peyman <i>et al</i>	$\sigma[S m^{-1}]$ difference (%)
0.001	5.02e-4 / 1.03e-3	1.03e-5 / 3.01e-5	2.05 / 2.93	0.011 / 0.012	0.009 / 0.009	-25.82 / -31.29
0.005	2.26e-3 / 4.26e-3	6.87e-5 / 1.53e-4	3.04 / 3.59	0.051 / 0.049	0.047 / 0.047	-8.42 / -4.36
0.01	4.21e-3 / 8.25e-3	9.85e-5 / 3.09e-4	2.34 / 3.75	0.095 / 0.095	0.094 / 0.094	-0.90 / -1.01
0.03	1.20e-2 / 2.30e-2	3.07e-4 / 9.04e-4	2.55 / 3.92	0.271 / 0.265	0.281 / 0.281	3.51 / 5.63
0.05	1.96e-2 / 3.90e-2	4.84e-4 / 1.81e-3	2.48 / 4.64	0.441 / 0.449	0.466 / 0.466	5.38 / 3.70
0.15	5.77e-2 / 1.18e-1	2.42e-3 / 1.50e-2	4.20 / 12.72	1.300 / 1.355	1.375 / 1.375	5.48 / 1.42

417

418 Table 1: Evaluation of amplitude accuracy. G [S] is the average conductance from 1 KHz to 400
419 KHz for different NaCl concentrations. The conductivity is calculated using the average
420 conductance and the cell constant. The values are given for both electrode configurations (needles
421 in straight line / needles in square).

422

423

NaCl [M]	Phase[°]	Phase[°] SD
0.001	5.66 / 6.96	7.78 / 7.89
0.005	2.00 / 2.79	1.80 / 1.25
0.01	1.35 / 2.29	0.78 / 0.65
0.03	0.95 / 1.79	0.28 / 1.26
0.05	0.81 / 1.69	0.55 / 2.24
0.15	2.07 / 6.14	1.43 / 6.59

424

425 Table 2: Evaluation of phase accuracy. The phase average and standard deviation from 1 KHz to
426 400 KHz for different NaCl concentrations. At this frequency range the phase should be
427 approximately zero, i.e., the measured phase is due to equipment errors and electrode artifacts and
428 should be minimized. The values are given for both electrode configurations (needles in straight
429 line / needles in square).

430

431

Frequency [Hz]	$\sigma[S\ m^{-1}]$ difference (%)	Phase[$^{\circ}$]
976.5625	3.30 / 4.06	1.84 / 6.66
1953.125	2.85 / 1.58	1.36 / 0.68
3906.25	2.38 / 2.08	1.07 / 0.44
7812.5	1.70 / 2.07	0.89 / 0.73
9765.625	1.58 / 2.99	0.95 / 1.06
15625	1.30 / 1.61	0.96 / 1.20
31250	0.45 / 1.81	0.90 / 1.89
65104	0.05 / 0.79	1.06 / 1.97
97656.25	-0.90 / 0.40	1.16 / 1.94
195312.5	-0.23 / -1.37	1.53 / 0.35
390625	-1.39 / -4.17	2.69 / 2.47

432

433

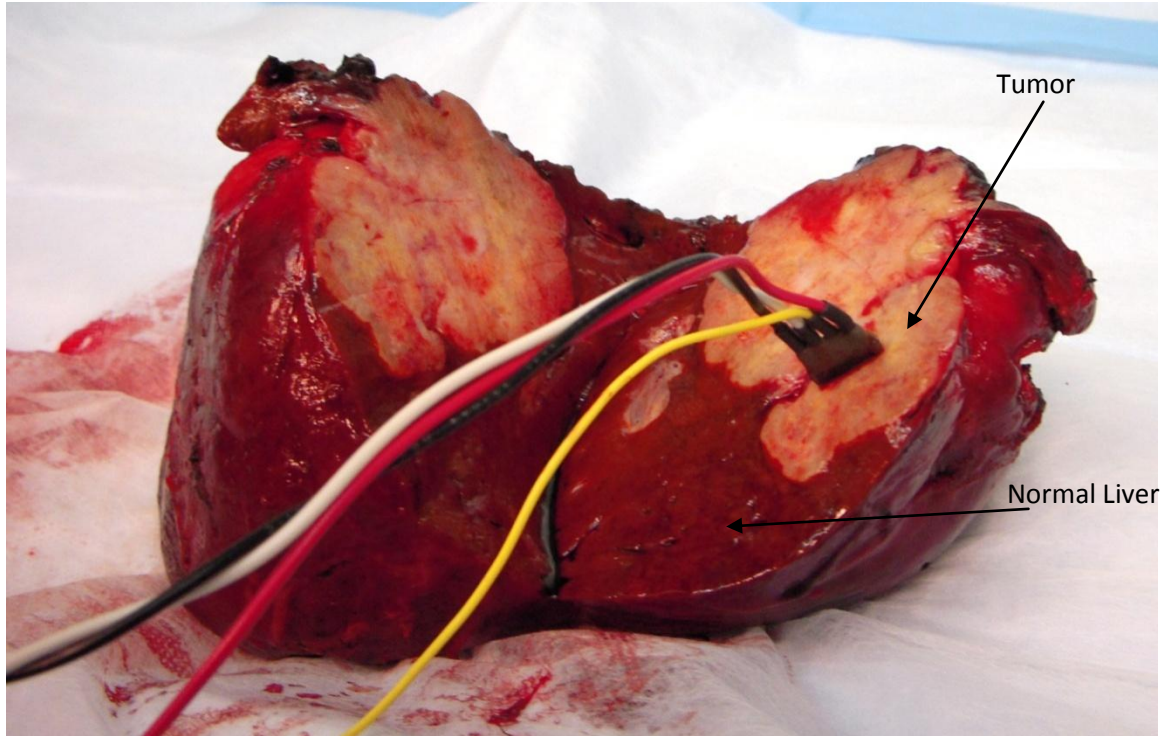
434

435

436

Table 3: For NaCl concentrations 0.005-0.15 M, for each frequency the average percentage of conductivity difference between the data obtained here and the literature and the average phase (which should be zero).

437
438
439
440



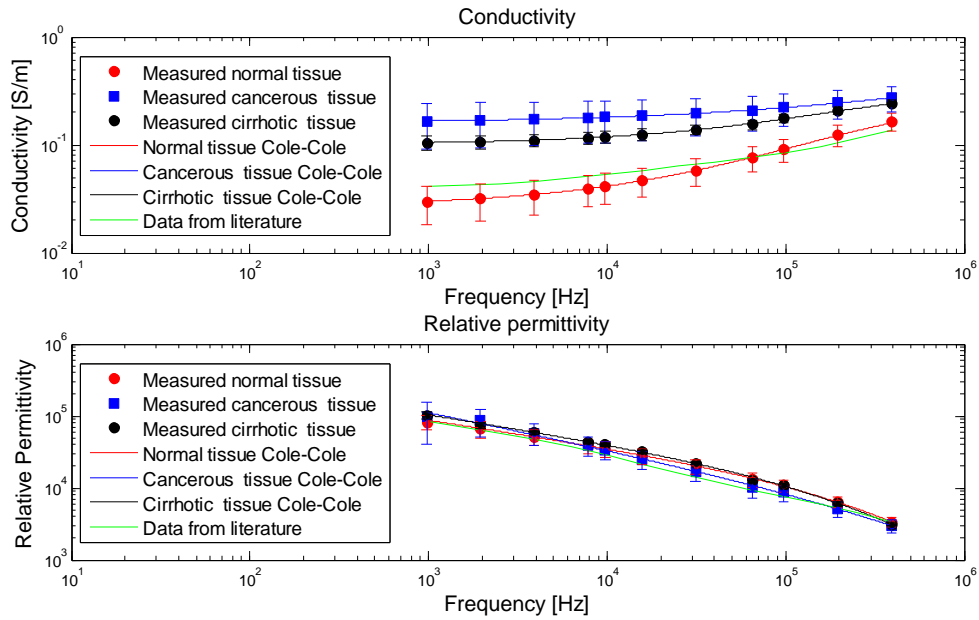
441
442
443

Figure 3 Example of measurement configuration.

Frequency [Hz]	Normal Conductivity [S/m]	Tumor Conductivity [S/m]	Cirrhotic Conductivity [S/m]	Normal Relative Permittivity	Tumor Relative Permittivity	Cirrhotic Relative Permittivity
976.6	0.030 ±0.01	0.166 ±0.08	0.105 ±0.02	8.2e4 ±2e4	9.9e4 ±6e4	1e5 ±1e4
1953.1	0.032 ±0.01	0.169 ±0.08	0.107 ±0.02	6.6e4 ±2e4	8 e4 ±4e4	7.4e4 ±6e3
3906.3	0.035 ±0.01	0.173 ±0.08	0.110 ±0.02	5.2e4 ±1e4	5.8e4 ±2e4	5.9e4 ±3e3
7812.5	0.040 ±0.01	0.179 ±0.08	0.116 ±0.02	3.8e4 ±9e3	3.9e4 ±1e4	4.5e4 ±2e3
9765.6	0.042 ±0.01	0.181 ±0.08	0.119 ±0.02	3.5e4 ±8e3	3.4e4 ±1e4	4e4 ±2e3
15625.0	0.047 ±0.01	0.185 ±0.08	0.125 ±0.02	2.8e4±7e3	2.5e4 ±7e3	3.2e4 ±1e3
31250.0	0.058 ±0.02	0.195 ±0.07	0.138 ±0.02	2e4 ±5e3	1.6e4 ±5e3	2.2e4 ±1e3
65104.0	0.076 ±0.02	0.209 ±0.07	0.155 ±0.02	1.3e4 ±3e3	1e3 ±3e3	1.3e4 ±7e2
97656.3	0.091 ±0.02	0.222 ±0.07	0.176 ±0.02	1.1e4 ±2e3	8.6e3 ±2e3	1.1 e4 ±5e2
195312.5	0.124 ±0.03	0.246 ±0.07	0.208 ±0.01	6.5e3 ±1e3	5.1e3 ±1e3	6.2e3 ±3e2
390625.0	0.164 ±0.03	0.272 ±0.07	0.244 ±0.01	3.4e3±5e2.	3e3 ±6e2	3.2e3±2e2

444 Table 4 Average conductivity and permittivity of normal, cancerous and cirrhotic tissues.

445



446
447

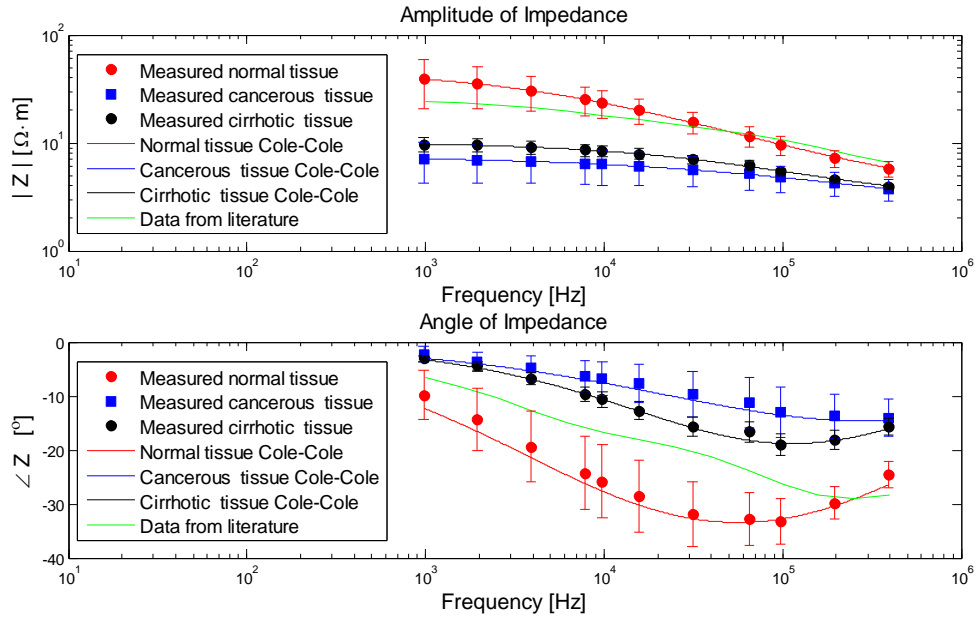
448 Figure 4: Average conductivity and permittivity of normal, cancerous and cirrhotic
449 tissues. As a reference, data from the literature are presented (Andreuccetti *et al.*, 1997;
450 Gabriel *et al.*, 1996b). The continuous lines are the Cole-Cole model fits for these data
451 with the following parameters:

452 Normal tissue: $\rho_0 = 37.26$ [Ohm-m] $\rho_\infty = 3.31$ [Ohm-m] $\alpha = 0.634$ $f_c = 9358$ [Hz]

453 Cancerous tissue: $\rho_0 = 6.23$ [Ohm-m] $\rho_\infty = 1.94$ [Ohm-m] $\alpha = 0.503$ $f_c = 144731$ [Hz]

454 Cirrhotic tissue: $\rho_0 = 9.9$ [Ohm-m] $\rho_\infty = 2.76$ [Ohm-m] $\alpha = 0.62$ $f_c = 41752$ [Hz]

455



456

457 Figure 5: Average impedance amplitude and angle of normal, cancerous and cirrhotic
 458 tissues. As a reference, data from the literature are presented (Andreuccetti *et al.*, 1997;
 459 Gabriel *et al.*, 1996b). The continuous lines are the Cole-Cole model fits for these data
 460 with the following parameters:

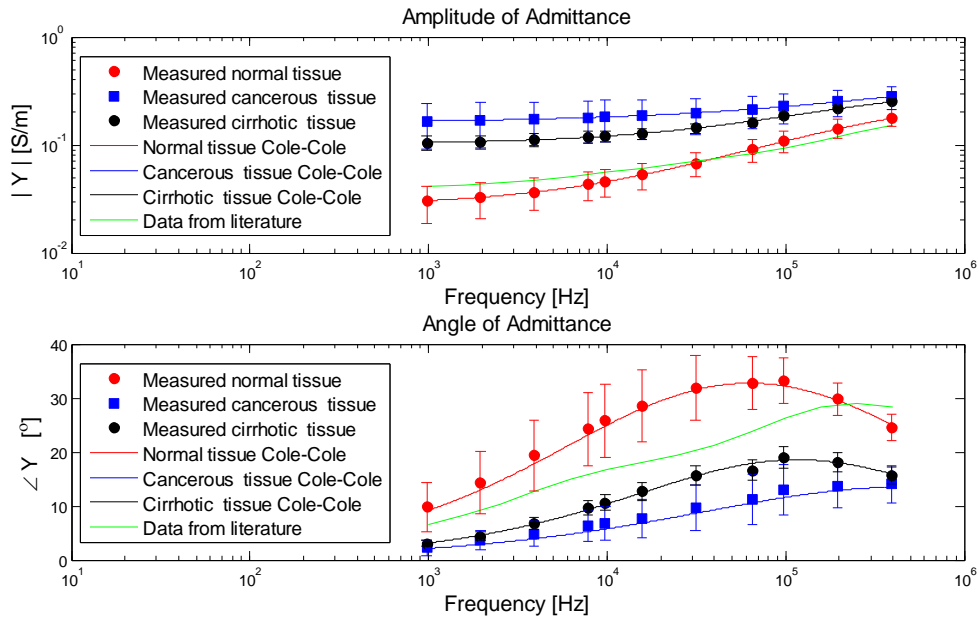
461 Normal tissue: $\rho_0 = 47.96$ [Ohm-m] $\rho_\infty = 3.06$ [Ohm-m] $\alpha = 0.594$ $f_c = 5567$ [Hz]

462 Cancerous tissue: $\rho_0 = 7.54$ [Ohm-m] $\rho_\infty = 2.04$ [Ohm-m] $\alpha = 0.492$ $f_c = 82294$ [Hz]

463 Cirrhotic tissue: $\rho_0 = 10.14$ [Ohm-m] $\rho_\infty = 2.76$ [Ohm-m] $\alpha = 0.617$ $f_c = 39682$ [Hz]

464

465



466
 467
 468
 469
 470
 471
 472
 473

Figure 6: Average admittance amplitude and angle of normal, cancerous and cirrhotic tissues. As a reference, data from the literature are presented (Andreuccetti *et al.*, 1997; Gabriel *et al.*, 1996b). The continuous lines are the Cole-Cole model fits for these data with the same parameters as in Figure 4.

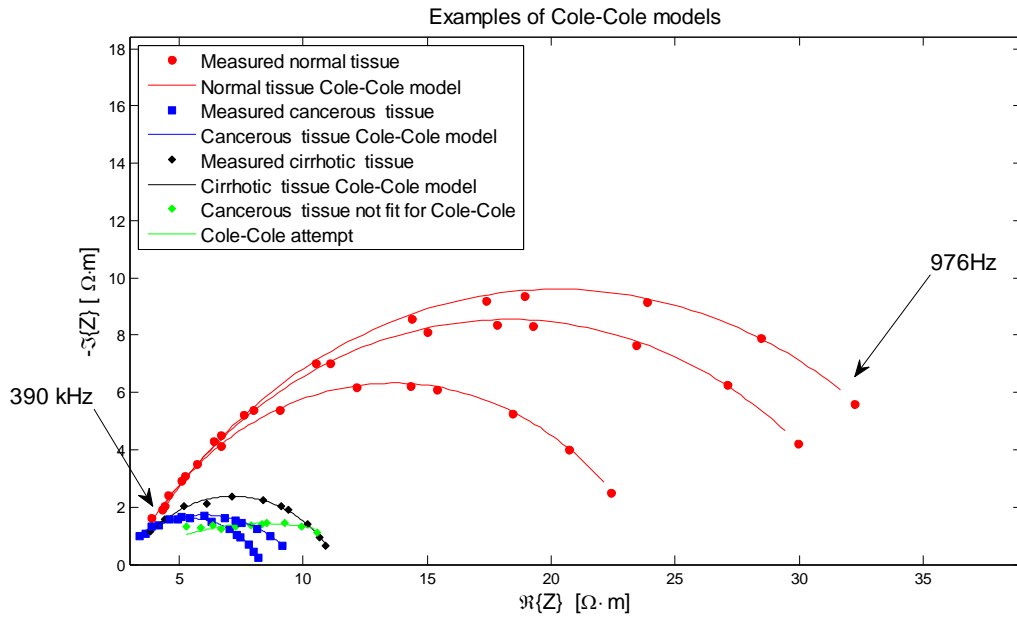
474

475

Parameters	units	Normal	Tumor	Cirrhotic
ρ_0	[Ohm-m]	47.58±28.94	7.56 ±3.44	10.14 ±1.66
ρ_∞	[Ohm-m]	3.29 ±0.65	2.15±0.72	2.76 ±0.14
α	-	0.63±0.04	0.51±0.14	0.62 ±0.02
f_c	[kHz]	10.5±7	155.7±132	41.5±8

476 Table 5: Average of the Cole-Cole parameters.

477



478

479

Figure 7: Examples of Cole-Cole models of different tissues.