Relationship between electrical admittivity and quantitative histopathology in human prostate

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Abstract. Passive bioelectrical properties have been demonstrated to provide sufficient contrast for use in differentiating benign from malignant tissue in a number of different organs including breast, prostate, cervix, bladder, and skin. The underlying microscopic anatomy responsible for these measured differences has been primarily speculative in the past. In this study we recorded electrical conductivity and permittivity spectra (100 Hz – 100 kHz) from 464 three mm diameter circular prostate samples. Each of these tissue specimens were stained with hematoxylin and eosin, processed onto microscopy slides, and digitized using optical microscopy. We used digital imaging processing tools to extract quantitative morphological features including total number of glands, average and total glandular lumen size, shape characteristics of the luminal spaces, and average and total glandular perimeter lengths. Correlative analysis was performed to assess the relationships between the tissue architectural features and the precisely co-registered electrical properties. We report on the findings from this analysis. This statistical assessment aims to provide a valuable piece of new information to help formulate a better understanding of the precise influence morphological architecture has on the flow of current through tissue.

1. Introduction
Several investigators over the last 20 years have verified that the electrical properties of malignant and benign tissues are significantly different and have demonstrated that measurement of these properties has obvious clinical potential. These findings have been confirmed for a number of different organ systems including breast [1], skin [2], cervix [3], bladder [4], and prostate [5], however the translation of this technology to the clinic has yet to be fully realized. The electrical property parameters used to describe tissues, including conductivity, permittivity, resistivity, admittance, impedance, and other model-base parameters are not part of the typical clinician’s vernacular. Further, these properties have been shown to differ for various pathologies, and hypothesis have been made regarding the biophysical mechanisms responsible for these differences, but no detailed analysis has been conducted to verify these hypothesis.

To address these issues we have instantiated a program aimed at quantitatively evaluating the relationship between bulk electrical properties and the underlying microscopic tissue morphology. The objective of this analysis is to provide electrical properties in terms of specific biological metrics that are readily appreciable by clinicians. While developing these relationships, a more complete understanding of the precise biophysical mechanisms responsible for observed electric properties will be realized. This early analysis reports specifically on the relationship between microscopic glandular components in prostate tissue and electrical admittance.
2. Methods

2.1. Electrical property sampling

Trans-admittance was recorded from 576 prostate samples using a custom-designed tetrapolar configured probe [6]. Specifically, conductivity and permittivity were sampled at 31 frequencies logarithmically-spaced spanning from 100 Hz to 100 kHz. The tissue samples are placed between two co-aligned circuit boards with coaxial electrode pairs printed on their surfaces. Drops of India ink are deposited into holes on both sides of the electrode pairs to identify the precise region probed. Straight pins are placed through these ink dots when the tissue has been removed from the probe. The tissue-pin combination is submerged in formalin for ~24 hrs to fix the tissue. Following fixation, 5 µm tissue samples are stained with H&E and microscopy slides are processed. The pin holes remain after the fixation process and provide visual landmarks to precisely delineate where electrical properties were recorded. A nonlinear least-squares approach [6] was employed to fit a Cole-Cole model to the 31 conductivity and permittivity values and the Cole parameters $\Delta \sigma$, $\sigma_\infty$, $f_c$, and $\alpha$ were extracted.

2.2. Digital image processing (DIP)

Each of microscopy slides were digitized with an Olympus BX51 (Center Valley, PA) microscope fitted with a EvolutionVF digital camera and motor-controlled three-axis stage controlled through ImagePro Plus software (Media Cybernetics, Bethesda, MD). Pin holes were manually identified and an array of high magnification (20x) sub-images encompassing these holes were sampled and automatically merged together to form a large-field composite image for analysis. The pin holes in each of the individual micrographs were identified manually and a mask denoting a circular region of interest representing the probes area of sensitivity was applied to the image. The green channel from the RGB images provided optimal contrast for distinguishing luminal spaces from background tissue and was selected as the processing channel. Image processing of the ROI included contrast enhancement, threshold-based segmentation to separate glandular luminal components from the remaining tissue matrix, and median filtering with a 5x5 kernel to eliminate pixel noise (see figure 1). 5 x 5 pixel kernels of approximately 300 different luminal areas were manually sampled to statistically estimate the threshold levels to use for luminal segmentation. Finally, luminal area, eccentricity, major axis length, minor axis length, and perimeter were computed for each of the individual objects identified. Objects with luminal areas less than 50 µm² were identified as artifacts and eliminated from the object list. Matlab’s Image Processing Toolbox was used for all digital image processing.

![Figure 1](image1.png)

**Figure 1.** Example digital image processing procedure. Arrows denote visible pinholes. Circular region in original image denotes the selected region of interest (ROI) which corresponds to the precise tissue area gauged with our transimpedance probe. Each of the objects detected denotes a single glandular element.
2.3. Statistical analysis
The sum, mean, and standard deviation of the luminal properties extracted from each ROI were computed. Linear correlation coefficients comparing the electrical properties and the DIP extracted luminal properties were calculated. We report specifically on the electrical property correlations with the total number of glands, total glandular area, mean glandular area, mean luminal eccentricity, mean length of major axis, mean length of minor axis, total sum of luminal perimeters, and mean luminal perimeter of the glandular objects identified within each ROI. Moderate significance was defined as p<0.05 and high significance was defined as p<0.001. All statistics were computed with Matlab.

3. Results
Of the 576 tissue regions probed and digitized, pin holes were clearly identifiable in 464 microscopy slides. A subset of the electrical parameters including conductivity and permittivity at 10 kHz and 100 kHz, and the four Cole parameters are presented as a scatter plot matrix in Figure 2 with the x-axes denoting the extracted luminal properties. Table 1 lists the correlation coefficients for each of the electrical parameters and luminal properties and notes which of these correlations are significant.

![Figure 2](image_url)

**Figure 2.** Scatter plots comparing the electrical properties to the extracted glandular properties. Conductivity and permittivity are plotted for 10 kHz only and are presented in units of S/m. The eight glandular properties displayed include the total number of glands, summed area of all luminal spaces, mean area of all glands, mean eccentricity of all glands, mean major axis length, mean minor axis length, summed perimeter of all glandular lumens and mean luminal perimeter from within each ROI. r values denote correlation coefficients with bolded entries representing significant findings (p<0.001).

| Electrical Properties | Frequency (kHz) | Total # | Total Area | Mean Area | Mean Ecc | Mean Maj Axis | Mean Min Perim | Total Perim | Mean Perim |
|-----------------------|----------------|---------|------------|-----------|----------|---------------|---------------|-------------|------------|
| σ                     | 0.1            | -0.29   | -0.28      | -0.04     | 0.04     | -0.06         | -0.09         | -0.32       | -0.1       |
|                       | 1              | -0.28   | -0.26      | -0.03     | 0.05     | -0.02         | -0.07         | -0.3        | 0.06       |
|                       | 10             | -0.22   | -0.19      | 0         | 0.06     | 0.01          | -0.01         | -0.21       | 0          |
|                       | 100            | -0.14   | -0.1       | 0.03      | 0.08     | 0.02          | 0.03          | -0.11       | 0.02       |
| ε                     | 0.1            | 0.19    | 0.2        | 0.04      | -0.06    | 0.05          | 0.1           | 0.22        | 0.11       |
|                       | 1              | 0.31    | 0.31       | 0.1       | -0.01    | 0.1           | 0.22          | 0.38        | 0.23       |
|                       | 10             | 0.38    | 0.36       | 0.08      | -0.05    | 0.05          | 0.19          | 0.42        | 0.15       |
|                       | 100            | 0.19    | 0.15       | 0.01      | 0.02     | -0.03         | 0.01          | 0.21        | 0.01       |
| σω                   |                | -0.02   | -0.06      | -0.04     | 0.07     | -0.03         | -0.05         | -0.02       | 0          |
| ∆σ                   |                | -0.14   | -0.09      | 0.02      | -0.03    | 0             | 0             | -0.16       | -0.05      |
| εω                   |                | -0.07   | -0.13      | -0.12     | 0.03     | -0.11         | -0.17         | -0.12       | -0.11      |
| α                    |                | -0.08   | -0.04      | 0.02      | 0.03     | -0.05         | -0.09         | -0.09       | -0.07      |

Table 1. Pearson correlation coefficients for digital image processing parameters and electrical properties. Total # denote the total number of glands detected within in an ROI, while all other parameters represent the sum of all or mean of all parameters computed from within a particular ROI. Bolded elements denote correlations reaching a level of significance p<0.05 and those bolded and shaded reached a significance of p<0.001.
4. Discussion

It is well-established that bioelectrical properties are a function of tissue morphology and the significant correlations observed here support these claims. In prostate, we have previously hypothesized [5-7] that the electrical properties are largely dependent on glandular density with more glandularly-dense tissues exhibiting higher impedances to current flow since charger carriers must traverse around these structures. Glandular lumens in benign prostate consist of a layer of tightly packed columnar-shaped epithelial cells that are lined by a ring of flat basal cells. These cellular structures inhibit low frequency currents from entering the luminal spaces and force current to flow around these spaces. Increases in glandular density provide a smaller area for current and result in a lower overall conductivity.

The significant negative correlations (p<0.001) noted between conductivity (0.1 – 10 kHz) and total # of glands, total luminal area, and total luminal perimeter support this hypothesis. These extracted luminal properties specifically tally the total amount of luminal space within a particular ROI. The other extracted parameters describe the characteristics of the average glandular structure within this ROI. For the most part, these average properties do not significantly correlate to the electrical properties; it is the global characteristics of the ROI that are the significant biological drivers dictating electrical properties. Of note, is the decreased conductivity correlation as frequency increases; this likely arises from the lower impedance cell membranes exhibited at higher frequencies.

In terms of permittivity, there is a significant (p<0.001) positive linear correlation noted between permittivity and total # of glands, total glandular area, and total luminal perimeters. The cell membranes of the epithelial and basal cells lining the glandular lumens provide a capacitive storage element for charge. The positive relationship noted between glandular content and permittivity suggests that with increased glandular density, cell membranes couple predominantly in parallel since capacitances sum in this configuration and permittivity is directly proportional to total capacitance.

Both $\Delta \sigma$ and $f_c$ were observed to be significantly correlated with the glandular morphology, while $\sigma_\infty$ and $\alpha$ did not demonstrate significant correlations. $\Delta \sigma$ is a combined measure of both high and low frequency conductivities and tracks the single frequency conductivity findings at 0.1 kHz and 100 kHz. $f_c$ is suspected to be largely cellular membrane dependent, however only moderately significant correlations were noted in this study. In a previous study [7], we have demonstrated that $f_c$ is significantly different in prostatic cancer tissues as opposed to benign tissues. This may suggest that $f_c$ is highly dependent on the type of cell and viability of that cell.

Additional image processing aimed at more precisely characterizing individual cell types, analyzing tissue texture, and quantifying specific tissue concentrations is suggested to more completely describe the relationships between tissue pathology and electrical properties.

5. Conclusion

The approach to assessing the relationship between electrical properties and tissue presented here is an important step toward understanding more completely the biophysical mechanisms response for certain electrical properties. This approach may yield an alternative method for reporting electrical properties so that instead of reporting in terms of electrical properties, we may be able to present doctors with information specifically regarding the pathological status of the tissue.

References

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