Towards mm-wave spectroscopy for dielectric characterization of breast surgical margins

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Abstract

Purpose

The evaluation of the surgical margin in breast conservative surgery is a matter of general interest as such treatments are subject to the critical issue of margin status as positive surgical margins can undermine the effectiveness of the procedure. The relatively unexplored ability of millimeter-wave (mm-wave) spectroscopy to provide insight into the dielectric properties of breast tissues was investigated as a precursor to their possible use in assessment of surgical margins.

Methods

We assessed the ability of a mm-wave system with a roughly hemispherical sensitive volume of 3mm radius to distinguish malignant breast lesions in prospectively and consecutively collected tumoral and non-tumoral ex-vivo breast tissue samples from 91 patients. We characterized the dielectric properties of 346 sites in these samples, encompassing malignant, fibrocystic disease and normal breast tissues. An expert pathologist subsequently evaluated all measurement sites.

Results

At multivariate analysis, mm-wave dielectric properties were significantly correlated to histologic diagnosis and fat content. Further, the mm-wave dielectric properties of neoplastic tissues were significantly different from normal breast tissues, but not from fibrocystic tissue.

Conclusion

Reliable discrimination of malignant from normal, fat-rich breast tissue to a depth compatible with surgical margin assessment requirements was achieved with mm-wave spectroscopy.

Highlights

- mm-wave dielectric properties of breast cancer differed from normal tissues.
- benign lesions had mm-wave dielectric properties of intermediate to normal and malignant tissues.
- mm-wave spectroscopy may have a role in surgical margin management.
- future studies should account for inter-individual differences in breast dielectric properties.

Keywords

surgical margin, tissue discrimination, millimeter waves, dielectric tissue properties

Abbreviations

mm-wave (millimeter wave), NBT (normal breast tissue), FCD (fibrocystic disease), MAL (malignant).

Introduction

Breast-conserving surgical management of breast cancer relies on quadrantectomy, lumpectomy and, more recently, on nipple sparing mastectomy [1,2]. Assuring the cancer is contained within the resected tissue requires a margin of healthy tissue between the outer limit of the tumor and the resection surface. Negative surgical margins are significantly correlated with lower rates of local recurrence [3,4] while positive margins have the potential to undermine procedure effectiveness. For ductal carcinoma in situ, it has been estimated that breast-conserving surgical procedures with a clear margin of 2 mm or more can be reliably considered as radical, while narrower margins are associated with progressively increasing local recurrence [5,6]. In this context, it has been estimated that 20-25% of breast cancer patients undergo re-excision due to positive or close (<2 mm) surgical margins, with consequent patient discomfort and increased costs [7-9]

A common strategy for minimizing the risk of positive surgical margins is intraoperative macroscopic assessment by a pathologist, with or without a frozen section examination of the resection margins closest to the neoplasm. Unfortunately, this requires the availability of a pathologist, increases pathology workload, and has high direct and indirect costs, stimulating research for alternative margin assessment strategies.

Several devices for intraoperative surgical margin assessment have arrived on the market, including destructive rapid evaporative ionization mass spectrometry (e.g. iKnife®, Waters Corporation, USA)[10]. Differences between the interactions of electromagnetic fields with malignant and normal tissues have been observed over a broad range of frequencies [11,12] and form the basis for non-destructive dielectric spectroscopy at radio (e.g. Marginprobe®, Dune Medical Devices, USA) [13-17] or terahertz frequencies (e.g. TPS Spectra3000®, Teraview Ltd., UK) [18]. Insomuch as the measured impedance of the tissue correlates with a “positive” or “negative” margin these devices can be used for margin evaluation.

For technological reasons, relatively little attention has been given to frequencies between 10 and 150 GHz (referred to as millimeter waves, or mm-waves). Relative to radio and microwaves, the shorter wavelength of mm-waves is of considerable interest for imaging systems because they should permit higher spatial resolution, while having greater penetration depths than higher frequency terahertz waves [19,20]. The penetration by mm-waves to depths of a few millimeters in human tissues with simple antennas may likewise present an interesting option for surgical margin testing.

In order to assess the ability of mm-waves to distinguish malignant breast lesions from surrounding breast tissues, we tested a mm-wave spectroscopy system on prospectively and consecutively collected tumoral and non-tumoral ex-vivo breast tissue samples.

Materials and Methods

Women undergoing breast cancer surgery were candidates for this ethics committee-approved study. All participants provided informed consent for measurements of dielectric tissue properties to be performed on the breast tissue excised in the course of their surgery.

Preparation of tissue samples

Immediately following excision, fresh specimens were delivered to the gross pathology room for standard gross examination. Between 1 and 3 (median and mode 1) parallelepipeds of tissue (7-10 mm width and height, 7-35 mm length), including breast lesions and healthy tissue, were isolated and shaped for measurement within a target of 90 minutes of excision, most of them in vacuum bags and at a temperature of 4° C.

mm-wave measurements

The system and procedures used for dielectric property measurements have been previously described [21,22]. In brief, it consists of a computer-controlled vector network analyzer (VNA) (E8361C, Keysight Technologies Italia, Milan, Italy) connected by a cable to a sealed coaxial antenna probe (Dielectric probe kit 85070E, Keysight Technologies Italia, Milan, Italy). The sensitivity of the probe tapers with depth and distance from axis of the probe [22], forming a roughly hemispherical sensitive volume of 2 mm radius (Figure 1), thus providing a close approximation of the needs of surgical margin assessment where a margin can be considered "clean" if tumor cells are not present to a depth of 2 mm.

Dielectric property measurements were then performed on 1 to 5 (median and mode 3) neighboring sites roughly 8 mm apart on each tissue sample. With the tip of the probe placed in contact with the tissue sample at a slight contact force, the VNA measured the dielectric properties at 1000 frequencies linearly spaced over the range from 0.5 to 50 GHz, and a computer recorded these values. The measurement was repeated five times for each site and averaged. The individual measurement sites were then inked to facilitate localization at histopathology (Figure 1a).

Histopathology evaluation

Full-face histology slides similar to those used for clinical histological margin evaluation (i.e. orthogonal to the cutting surface; Figure 1b) were subsequently obtained oriented perpendicular to the surface of contact with the mm-wave probe on a mid-plane of the sensitive volumes. A pathologist with 20 years experience in breast tissue histopathology assessed the slide for the presence of breast lesions and measured the distance of any identified lesion from the surface in contact with the mm-wave probe.

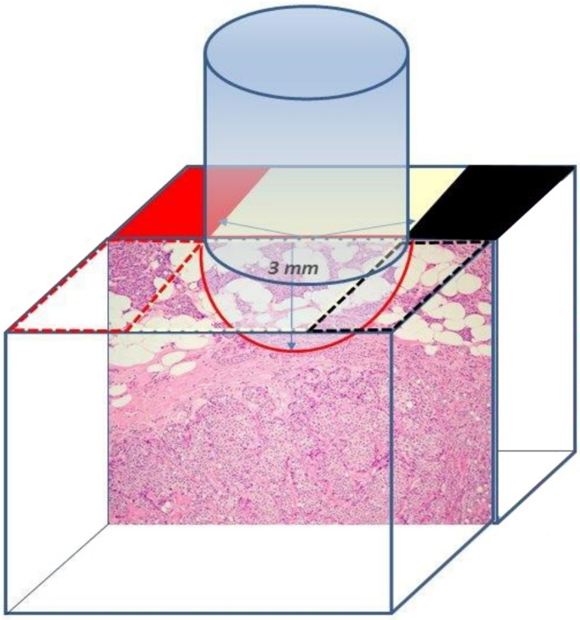

Figure 1. Each parallelepiped tissue sample was placed in contact with the mm-wave probe (grey cylinder) and dielectric property measures performed over the 0.5 – 50 GHz range. One to five sites were subjected to measurement on each parallelepiped and then inked with a distinct color (shown here as red, yellow and black). After fixation, full-face histology slides (inset) were prepared perpendicular to the surface of measurement and passing through the centers of the inked regions.

For each measurement site, breast tissue contained within the estimated sensitive volume of the mm-wave probe was histologically assessed and classified into three categories: normal breast tissue (NBT), fibrocystic disease (FCD), and malignant (MAL). The MAL category encompassed both invasive and non-invasive lesions, as both are grounds for resection widening by the surgeon.

Clinical measurement and pathological parameters recorded for their possible influence on the measurements were: age, menopausal status (pre- post-), time to measurement after surgery, distance in µm between the tissue surface (device probe) and the pathologic lesion as seen at pathology (binarized as direct contact / no contact), cellularity, histologic aspect (variegated versus homogenous), percentages of adipose, and of fibrous or epithelial tissues. In case of malignant lesions, pathological characteristics recorded included tumor size, grade, istotype, growth pattern classification [23], nodal status, and immunohistochemical expression of estrogen receptors, progesterone receptors, HER2/NEU and Ki-67 labeling index, but are not examined in the statistical analysis herein.

The data acquired for each measurement site were fitted (Matlab 2016, Mathworks, Natick USA) to a single-pole formulation of the Cole-Cole model for dielectric permittivity [12] to obtain 5 parameters: e_s, e_∞ (the tissue permittivity at extremely low and high frequencies respectively); σ (the tissue conductivity); τ (a relaxation time for recovery from changes to the electric field in the tissue); and α (a fitting term describing the dependence of permittivity on change in frequency). Fitting quality was assessed by residual errors to ensure that all results were physically plausible.

Statistical analysis

Statistical analyses were carried out using R software (ver. 3.3, R Foundation for Statistical Computing, Vienna, Austria)[24]. In preparation for the evaluation of diagnostic performance, we first evaluated whether, and which Cole-Cole parameters vary with diagnosis, tissue characteristics, and patient demographics, by Bayesian multivariate generalized linear mixed models with Gaussian error distribution using the MCMCglmm package in R [25]. The patients were considered a random effect as multiple sites were measured in each patient.

Reflecting the clinical priority of ensuring malignant sites are recognized, we subsequently, assessed the capability of Cole-Cole parameters to classify samples as malignant (MAL) vs non-malignant (NBT + FCD). In consideration of the findings in the random effects analysis, the classification was conducted with a Bayesian logistic model (MCMCglmm with binomial error distribution), considering the patient as a random effect. The output variable in this approach is the probability that a sample is malignant. The logistic model was validated using cross-folding: the set of samples was divided at random into 5 equal-sized sub-sets, each having the same distribution of malignant/healthy samples; and in turns, 4 subsets were used for generation of the model and the fifth used for testing. A receiver operator curve (ROC) assessment was performed to evaluate the overall ability of the model to correctly identify MAL relative to the other two groups. Additional ROC assessments of classification performance were carried out for the distinction between pairs of subgroups: MAL vs NBT, FCD vs NBT, and MAL vs FCD.

Results

124 female patients who had been scheduled for breast surgery were recruited to this study. In 26 cases, the time between excision and initiation of gross pathology preparation exceeded the maximum time post-excision of 4 hours, and in seven the tissue samples were too small and did not proceed to measurement. In the remaining 91 patients, dielectric properties and histopathology were obtained for 346 breast tissue sites, encompassing a broad spectrum of the most common breast lesions. A summary of patient, and tumor characteristics is given in Table 1.

None of the Cole-Cole parameters were significantly associated with menopausal status, cellularity, percentage of fibrous or epithelial tissues, overall aspect or the distance of lesion from the mm-wave probe (see Supplementary Figure 1).

At multivariate analysis, none of the clinical and pathological characteristics other than fat content and histologic diagnosis were significantly correlated with Cole-Cole parameters at multivariable analysis. Only two Cole-Cole parameters, namely e_s and σ, were seen to be significantly correlated with histologic diagnosis ($p<0.05$) and fat content ($p<0.001$) (see Supplementary Figure 2 left panel and Supplementary Table 1).

Notably, we found significant variability in the Cole-Cole parameters between patients (random effect: $p < 0.001$) that was not strictly related to histopathologic category (see Supplementary Figure 2, right panel) and accounted for between 3.4% and 11.9% of the total variance in the parameters. The Cole-Cole parameter τ was the least affected by patient. The MAL samples had Cole-Cole coefficients significantly different from those associated with NBT ($p = 0.006$), but not significantly different from FCD ($p = 0.064$).

Table 1. Summary of Patient Demographics, and Sample Characteristics

Characteristic	Patients	Tissue Subgroups		
		NBT	FC	MAL
Patients (#)	91			
Age (mean (range))	53.8 (28-80)yrs			
Post-Menopausal (#,(%))	37 (40.9%)			
Time Post-Excision^ (mean (range))	85 (10-228)min			
Measurement Sites (#)	346	97	142	107
Fat Content (mean (s.d))		94.7 (0.8%)	28.7 (2.6%)	10.1 (1.8%)
Direct Contact (#,(%))		85 (87.6%)	121 (85.2%)	83 (77.6%)

^ if not examined within 20 minutes, the samples were stored in vacuum bags at 4° C, maximum allowed time post-excision was 4 hours.

The logistic model showed that Cole-Cole parameters could reliably (AUC = 0.776 ± 0.030) distinguish malignant (MAL) from benign (NBT + FCD) tissues (Figure 2a, Table 2), with high sensitivity (0.869) and moderate specificity (0.574), corresponding to a low false negative rate (0.131) and moderate false positive rate (0.426); with the false positives being dominated by fibrocystic tissues.

The classification of pairs of subgroups fully supported these findings (Figure 2b): MAL and FCD were both distinguished from NBT with high degrees of accuracy ($AUC_{MAL\ vs\ NBT}$: 0.919 ± 0.016; $AUC_{FCD\ vs\ NBT}$: 0.860 ± 0.021), whereas the distinction between MAL and FCD was clearly lower ($AUC_{MAL\ vs\ FCD}$: 0.681 ± 0.048).

Table 2. Diagnostic performance of mm-wave dielectric measurements for breast tissue discrimination

	AUC	Sensitivity	Specificity	PPV	NPV
MAL vs (FCD + NBT)	0.776 ± 0.030	0.869	0.574	0.131	0.426
MAL vs NBT	0.919 ± 0.016	0.930	0.860	0.070	0.140
FCD vs NBT	0.860 ± 0.021	0.850	0.796	0.150	0.204
MAL vs FCD	0.681 ± 0.048	0.775	0.589	0.025	0.411

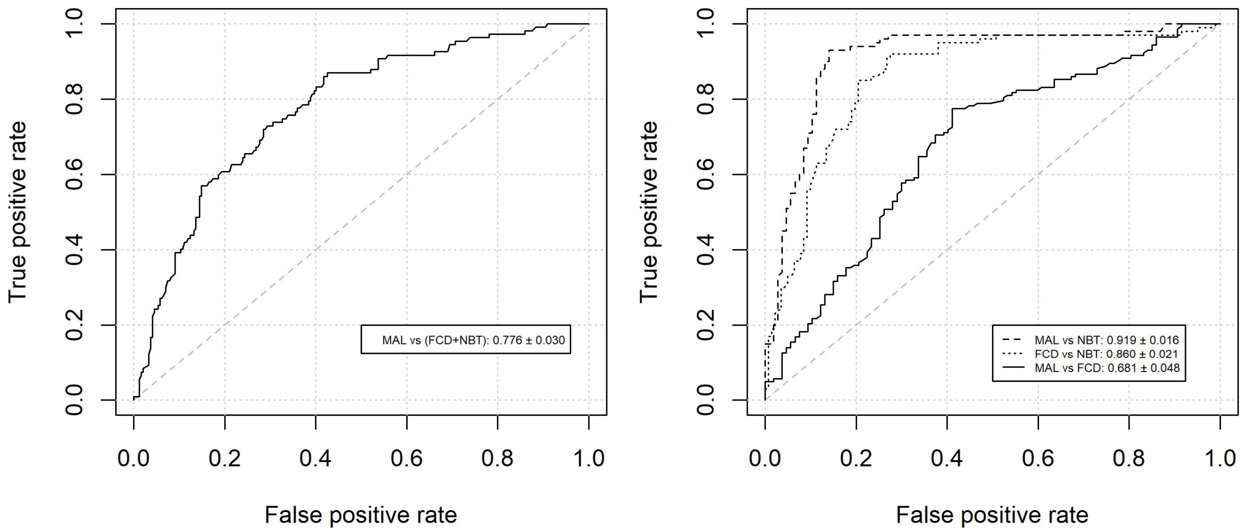

Figure 2. a) The overall differentiation between malignant (MAL) and non-malignant (FCD + NBT) tissues gave an AUC of 0.776. b) Comparing ROC curves for tissue subgroups; differentiation between MAL and the FCD subgroup had an AUC of 0.919 (dashed line) while between MAL and FCD the AUC was 0.681 (solid line). The differentiation between FCD and NBT was intermediate between the others, with an AUC of 0.860.

Discussion

Our results extend previous reports of the potential for diagnostic use of tissue dielectric properties in discriminating breast lesions to the mm-wave frequencies. In particular, using a probe whose sensitivity profile adheres to recent guidelines on clear margin depth in conservative breast surgery, our accuracy in distinguishing malignant from normal breast tissue was high (AUC 0.919), and good with respect to non-malignant tissues generally (AUC 0.776).

In evaluating a closely related approach to margin assessment, namely the radio-frequency-based MarginProbe® device, Kaufman et al. [17] have reported performing 4322 measurements on 106 samples. The differentiation between malignant lesions and fat-dominant mammary tissue was characterized by an ROC curve with AUC of 0.96, and sensitivity and specificity of 90 and 91% respectively. This is very similar to our performance between malignant and normal breast tissues (MAL vs NBT), and somewhat better than we obtained for malignant vs non-malignant tissues (MAL vs NBT + FCD). It should be noted however, that the performance in that study is likely to be overestimated due to the non-independence of the samples following the large number of measurements replicated within each patient. Equally, the fraction of tissue samples in that study that did not correspond to malignant or healthy breast tissue (i.e. FCD) was far lower than the fraction of fibrocystic tissues in the present our study. As the parallelepipeds of tissue prepared for our study were selected based on gross pathology assessment to contain a mixture of normal and abnormal tissue for each patient, it may be that we have over-represented fibrocystic disease with a resultant reduction in the performance of distinguishing malignant vs non-malignant tissues (MAL vs NBT + FCD) This represents one of the principle limitations of our study. As a further note, Kaufman et al. [17] performed pathology assessment on a tissue plane parallel to the cut surface and thus may have underestimated the presence of malignant or fibrocystic tissues within the 2mm margin thickness but outside the examined plane.

As seen previously at lower frequencies, we found two of the five parameters involved in the Cole-Cole model dielectric properties (e_s and σ) to be strongly correlated to fat content [11,12]. This likely explains the lower performance in distinguishing malignant from fibrocystic disease (AUC 0.681) as a high density of cells and interstitial fluid characterizes both and consequently a low fat content compared to normal breast tissue. To improve performance in this area, it is likely to be necessary to go beyond dielectric properties alone, and to incorporate additional independent information about the tissues. A number of options exist for doing so, including pre-operative mammography or ultrasound to provide densitometry characteristics of breast tissue, coupling the mmWave probe to a device for elastographic characterization of pseudotumoral sclerosing lesions of intra-operative use, or limiting gross intraoperative evaluation with or without frozen section analysis to these uncertain cases.

It remains to verify our findings in the intra-operative context, using fresh, ex-vivo breast tissue and comparison with the final histopathology findings on a per-resection basis both with and without intraoperative macroscopic assessment by a pathologist. Nonetheless, our experience leads to a couple of considerations for future developments. First, our finding of significant inter-patient variability in the Cole-Cole parameters due to the patients themselves (i.e. not related with the disease) has implications for the choice of statistical analysis applied to classification of tissues. Experimental designs in future should examine replicate sampling within subjects to control for among-subject variability in response to treatments before generalized results to patients beyond those involved the experiment. At the same time, the patient specific effect raises the possibility that diagnostic performance can be improved by adjusting the decision criteria on the basis of measurements on fatty tissues of the individual patient.

Second, despite a number of devices, putatively able to guide the surgeon during breast resection already being available so far, none of them has had a significant clinical or commercial impact. The system used in this study can be subjected to extreme simplification of electronics, which should allow the device to be cost-effective not only in hospitals that are not equipped to perform intraoperative pathology, but also in aiding a pathologist to choose the areas of interest to submit to the frozen section. The system allows assessment of individual points on a tissue surface in a few seconds and should Also to be examined is whether the sensitivity to pathology extends to surgical contexts beyond breast cancer.

In conclusion, we have demonstrated that the diagnostic potential of dielectric properties extends to the mm-wave frequencies using a system that adheres to recommendations for evaluation of surgical margins in breast cancer. Translation to use in evaluation of the surgical margin in breast conservation surgery will require validation of our results in the intra-operative setting, and the design of a low-cost device that allows rapid margin assessment while maintaining performance in discriminating tumor foci.

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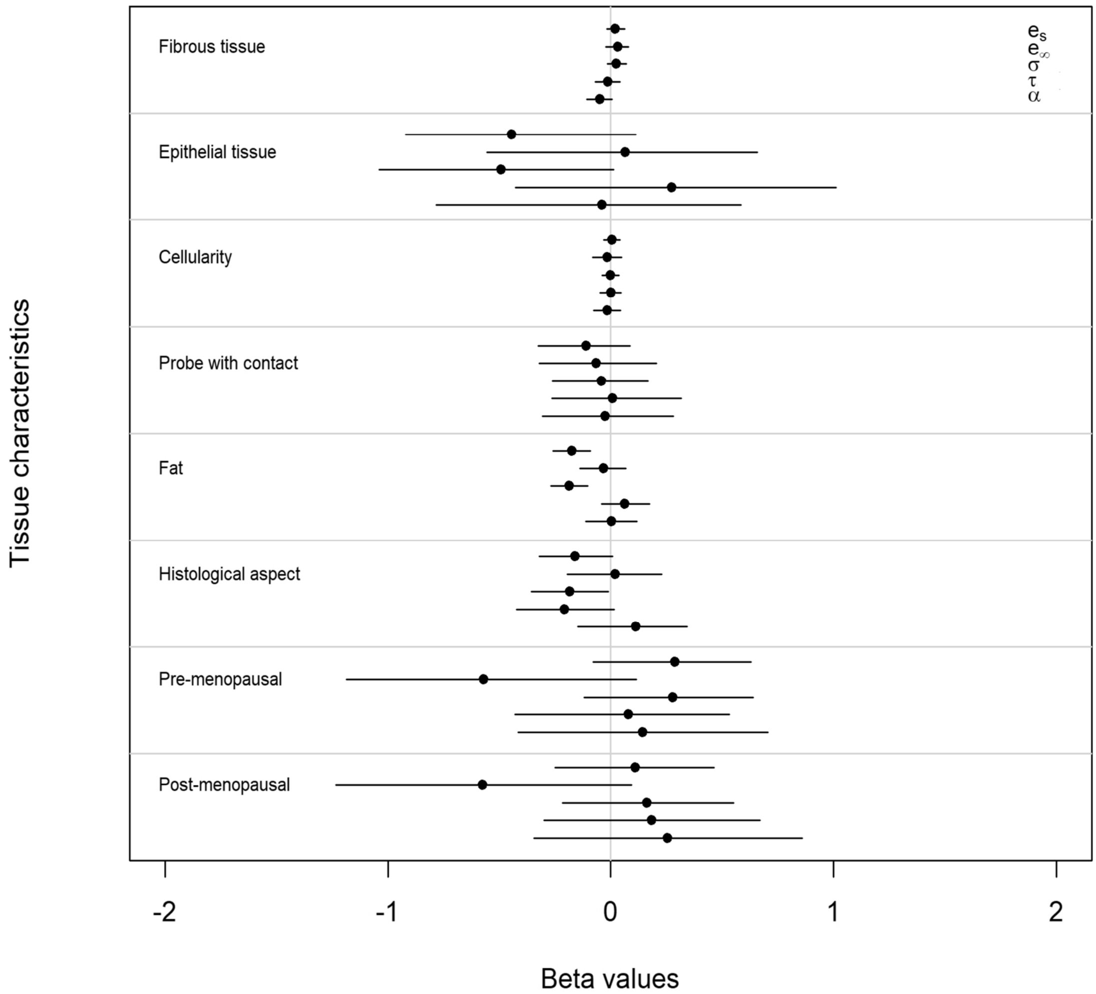
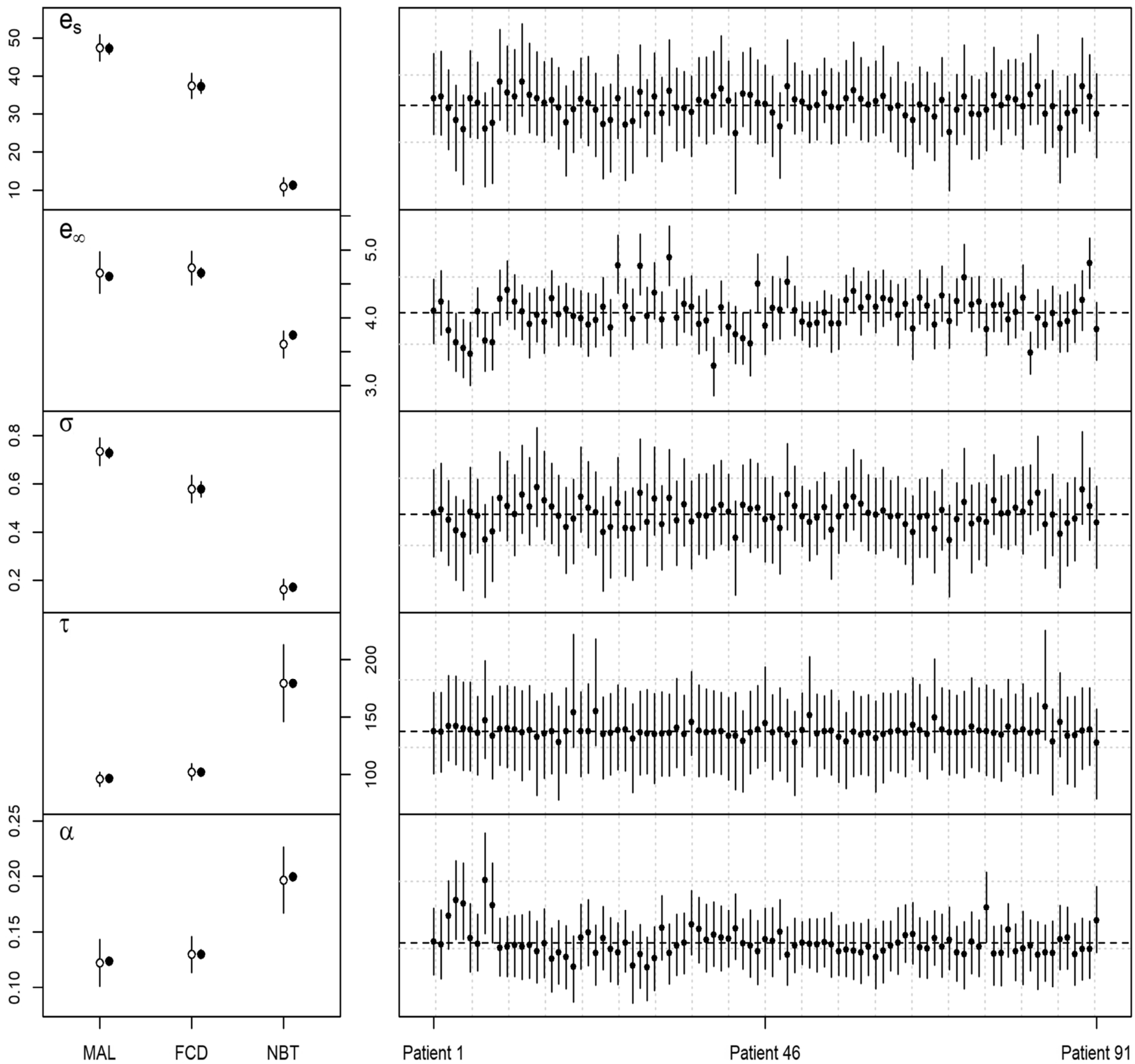
Supplementary Material



Supplementary Figure 1. Fixed effects analysis for the influence of tissue characteristics on the five Cole-Cole parameters fitted by Bayesian multivariate generalized linear mixed models (MCMCglmm). Horizontal lines segments represent 95% confidence intervals (CIs). Values for fat, cellularity and fibrous tissue have been magnified 10 times for graphical reasons. In all cases the 95% CIs include the zero value, indicating a non-significant effect of the tissue characteristics on the Cole-Cole parameters.



Supplementary Figure. 2. Results of fixed and random effects models fitted by Bayesian multivariate generalized linear mixed models (MCMCglmm). The left panels show the observed means and standard deviations (white circles and bars) of Cole-Cole parameters (from top to bottom: e_{∞}, e_s, τ, σ and α) along with their fitted values in the fixed effects model (filled circles) in malignant (MAL, n=107), fibrocystic disease (FCD, n=142), and normal breast tissues (NBT, n=97). The right panels show the variation between individual patients in the Cole-Cole parameter estimates according to the random effects model. The horizontal dashed line represents the group average. Vertical line segments represent 95% confidence intervals about the patient-wise mean fitted value. Notably, an unexpectedly large number of subjects lay outside this interval for e_{∞}, τ, and α.

Supplementary Table 3. Significant associations of Cole-Cole parameters with diagnosis and fat content.

Cole-Cole Parameter	Final Diagnosis	Fat content
e_s	*	***
e_∞	n.s.	n.s.
σ	*	***
τ	n.s.	n.s.
α	n.s.	n.s.