Buenos Aires – 5 to 9 September, 2016
Acoustics for the 21st Century…

PROCEEDINGS of the 22nd International Congress on Acoustics

Biomedical Acoustics: Paper ICA2016-647

Evaluating margin status with high-frequency (20-80 MHz) analytical ultrasound during breast conservation surgery

Robyn Omer[(a)], Amy LaFond[(a)], Caitlin Carter[(a)], Leigh Neumayer[(b)], Rachel Factor[(c)], Timothy Doyle[(a)]

[(a)] Utah Valley University, United States, timothy.doyle@uvu.edu
[(b)] University of Arizona, United States, lneumayer@surgery.arizona.edu
[(c)] University of Utah, United States, rachel.factor@path.utah.edu

Abstract

A majority of patients with early stage breast cancer elect breast conservation surgery (BCS) since it preserves unaffected breast tissue and, when followed by radiotherapy, provides survival rates equal to those of mastectomy. To ensure all of the cancer has been removed, excised tissue is sent to a pathology lab for analysis to determine if residual cancer is present in the margins (the boundary between resected and unresected tissue). This analysis can take up to several days. Unfortunately, 30-60% of BCS patients undergo additional surgeries to excise residual cancer not identified and removed during the initial surgery. The development of an intraoperative method to evaluate resected margins is, therefore, a crucial priority in breast cancer therapy. The objective of this work was to determine the sensitivity and specificity of high-frequency (20-80 MHz) analytical ultrasound for detecting residual cancer in margins by conducting a 17-patient pilot study and 73-patient validation study at the Huntsman Cancer Institute, Salt Lake City, Utah. Point measurements at 775 positions were collected from 383 resected margin specimens in through-transmission mode using 50-MHz, 6.35-mm diameter, single-element transducers. Attenuation and peak density (the number of peaks and valleys in a specified frequency band) were calculated from the ultrasonic waveforms and power spectra, respectively, and the two parameters were combined to perform a multivariate analysis. The pilot and validation studies showed sensitivity values of 87.5% and 87.0%, respectively, and specificity values of 82.9% and 67.2%, respectively. The results demonstrate that high-frequency ultrasound provides excellent sensitivity and good specificity for the rapid, intraoperative evaluation of BCS margins.

Keywords: High-frequency ultrasound, breast cancer, margins

Evaluating margin status with high-frequency (20-80 MHz) analytical ultrasound during breast conservation surgery

1 Introduction

Breast cancer is the second most prevalent cancer for women and has been historically treated with mastectomy to remove the entire breast. Breast conservation surgery (BCS) followed by radiotherapy is a less invasive approach that preserves the non-cancerous portion of the affected breast while providing survival rates similar to those of mastectomy. Consequently, a majority of patients with early stage breast cancer now select BCS. To ensure all of the cancer has been removed, surgeons send excised tissue to a pathology lab for analysis that can take several days. Detection of cancer in the margins of tissue samples (the boundary between resected and unresected tissue) indicates that some of the cancer was missed during BCS and the patient must return for additional surgery. Unfortunately, 30-60% of BCS patients undergo a second or third surgery to excise residual cancer not detected during the initial surgery [1]. A number of methods are therefore being investigated for the rapid, noninvasive evaluation of margin status in the operating room [2-5].

In 2010, a pilot study was conducted at the Huntsman Cancer Institute (HCI), Salt Lake City, UT, to determine the potential of high-frequency (HF) ultrasound (20-80 MHz) for detecting malignant tissue in excised surgical specimens during BCS [6]. In this study, 53 positions were tested on 34 excised specimens from 17 patients. The parameters derived from the ultrasonic signals were peak density and attenuation. Peak density is the number of peaks (maxima) and valleys (minima) in the power spectrum of the HF signal. The pilot study revealed that peak density correlated with breast tissue pathology. An initial analysis of the study data showed that peak density most closely matched five broad tissue categories corresponding to (1) fat necrosis, fibroadenomas, and tubular adenomas; (2) normal tissue; (3) atypical pathologies including benign calcifications, atypical ductal hyperplasia, fibrocystic changes, and benign papilloma; (4) ductal carcinomas, *in situ* and invasive; and (5) lobular carcinomas, *in situ* and invasive. Attenuation showed less of a correlation to these five pathology categories.

In 2013-2014, a validation study was conducted at the HCI to gather more data to verify the detection capabilities of HF ultrasound with high statistical significance. In this study, 722 positions were tested on 349 surgical margins from 73 BCS patients. Previous analysis of the pilot study data focused on the ability to differentiate between the five pathology categories, for example, with a one-way ANOVA test [6]. The present paper reports on analysis of the pilot study and validation study data using a binary classification test (Fisher's Exact test) with respect to malignant versus nonmalignant indications.

2 Methods

2.1 Specimen Testing

Ultrasonic testing of tissue specimens was performed during the course of routine BCS at the HCI in accordance with the ethical principles and guidelines for human subjects research. These included approval by the University of Utah Institutional Review Board (IRB 00037350 and 00062775) and informed consent from patients. Specimens ranged from 1-5 cm in length and width, 0.2-1.5 cm in thickness, and did not require any additional procedures or resection that affected the patient or surgical outcome. Immediately following resection, each specimen was placed inside a resealable plastic storage bag for ultrasonic testing and labeled with a de-identified specimen number. During ultrasonic testing, the outside of the bag was coupled to the ultrasonic transducers with ultrasound scanning gel (Sonotech® Clear Image for the pilot study and glycerol for the validation study). One to five positions were tested on each specimen depending on specimen size. Triplicate waveforms were acquired from each test position. Routine pathology was performed on the specimens after ultrasonic testing and the findings were correlated to the ultrasonic results.

Through-transmission measurements were acquired from the specimens with the use of a HF ultrasound system consisting of two 50-MHz immersion transducers (Olympus NDT, V358-SU, 12.7-mm OD, 6.35-mm active element diameter), a HF square-wave pulser/receiver (UTEX, UT340), and either a 500-MHz digital oscilloscope for the pilot study (Hewlett-Packard, HP-54522A) or a 1-GHz digital oscilloscope for the validation study (Agilent, DSOX3104A). An aluminum test fixture supported the tissue specimen and held the transducers in contact with the sample. Figure 1(a) is a close-up of the aluminum test stage and measurement configuration for the surgical specimens (scanning gel and resealable plastic bag not shown). Ultrasonic waveforms were averaged in the signal acquisition. Specimen thickness was recorded for each measurement.

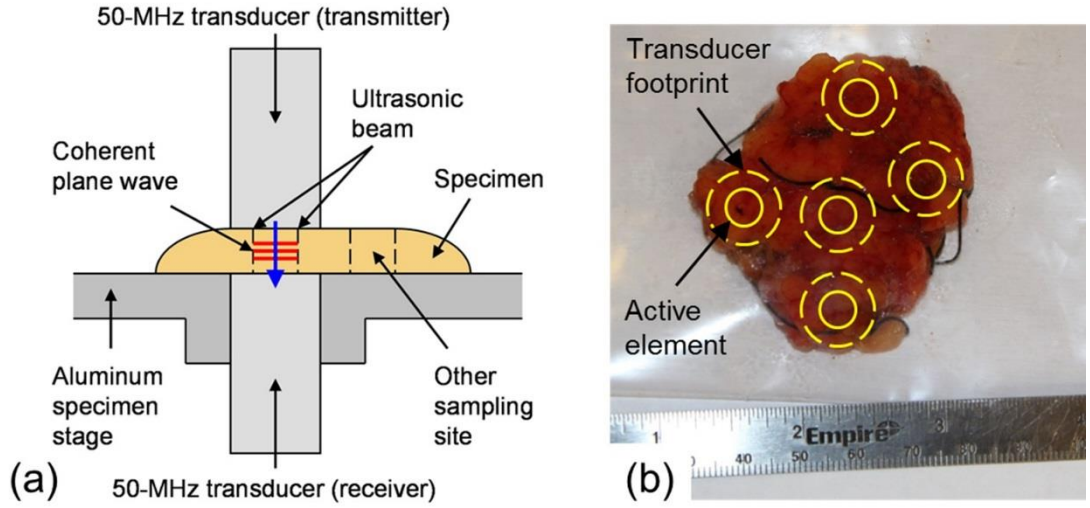

Figure 1: (a) Measurement configuration for surgical specimens. (b) Surgical specimen showing measurement positions.

Because of the small wavelength of the transmitted ultrasound (30 μm for 50 MHz) in comparison to the size of the active element (6.35 mm), the transmitting transducer produced coherent plane-wave pulses which propagated through the tissue to the receiving transducer. Figure 1(b) is a photograph of a BCS specimen inside a resealable plastic bag for ultrasonic testing in the validation study. The yellow circles illustrate the amount of tissue that the transducer positions sampled on the specimens. The transducer footprint (overall transducer area) and active element area (tissue sampling region) are shown as dashed and solid circles, respectively.

Peak densities were derived from the time-domain waveforms by performing a Fourier transform and counting the number of peaks and valleys in the 20-80 MHz region of the resulting power spectra [Figure 2(a)]. Attenuations were calculated by scaling the waveforms to account for receiver gain and tissue thickness, and using a Hilbert transform to obtain the waveform envelopes and their corresponding amplitudes [Figure 2(b)].

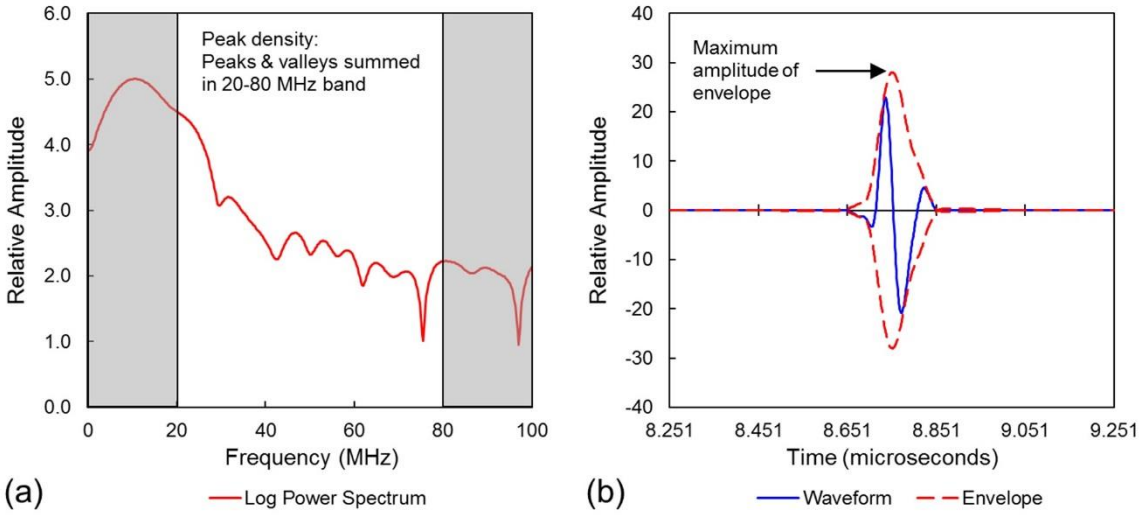

Figure 2: (a) Peak density measurement of a power spectrum. (b) Attenuation measurement based on waveform envelope amplitude.

2.2 Statistical Analysis

An optimization method was used to calculate statistical measures for peak density and attenuation. Since malignant tissues produced higher peak densities and attenuation coefficients than nonmalignant tissues, thresholds were defined to classify specimens as either malignant (parameter above threshold) or nonmalignant (parameter below threshold). To optimize the threshold for each parameter, an analysis was used that incrementally varied the threshold. The accuracy, sensitivity, and specificity were then calculated and plotted as a function of the threshold using Fisher's Exact test and HCI pathology findings as the gold standard. For the pilot study, final thresholds were obtained from those that gave the highest accuracy values. For the validation study this approach did not work well and yielded very low sensitivities. Instead, final thresholds for the validation study were chosen based on those values that gave comparable levels of accuracy, sensitivity, and specificity.

A multivariate analysis was also performed that combined the peak density and attenuation. Figure 3 shows the procedure applied to the pilot study data. In Figure 3(a), specimen coordinates were plotted as a function of peak density (x-axis) and attenuation (y-axis). The data points from malignant specimens tended to cluster in the upper right corner of the plot in Figure 3(a). To isolate the malignant specimens from the nonmalignant specimens, the coordinate system was translated and rotated. A parabola was then used to separate most of the malignant specimens from the nonmalignant specimens, as displayed in Figure 3(b), and a Fisher's Exact test was performed to obtain the accuracy, sensitivity, and specificity of the analysis approach.

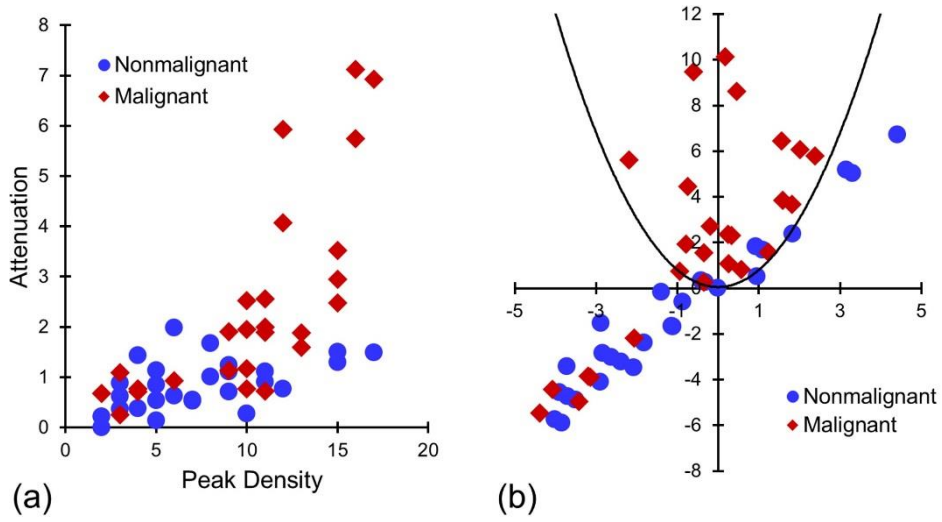

Figure 3: Multivariate analysis of peak density and attenuation with (a) original axis orientations and (b) transformed axis orientations.

For the multivariate analysis of the validation study, the malignant specimens were best isolated by not rotating and translating the coordinate system as executed in the pilot study (Figure 3). Instead, specimen coordinates were plotted as a function of attenuation (x-axis) and peak density (y-axis). A parabolic-like region was then defined with an inverse power law curve as a function of attenuation and with a high attenuation cut-off at a value of 1.5.

3 Results

3.1 Optimization of Statistical Measures

Figure 4 shows the optimization of the statistical measures for the pilot study, with Figures 4(a) and 4(b) showing peak density and attenuation, respectively. For peak density, a threshold of 9.5 maximized the accuracy at 71.7%. For attenuation, a threshold of 1.8 maximized the accuracy at 77.4%. The multivariate analysis for the pilot study yielded an accuracy 81.1%. Figure 5 shows the optimization of the statistical measures for the validation study, with Figures 5(a) and 5(b) showing peak density and attenuation, respectively. For peak density, a threshold of 8.25 maximized the accuracy at 71.1%. For attenuation, a threshold of 1.0 maximized the

accuracy at 60.0%. The multivariate analysis for the validation study yielded an accuracy 72.7%.

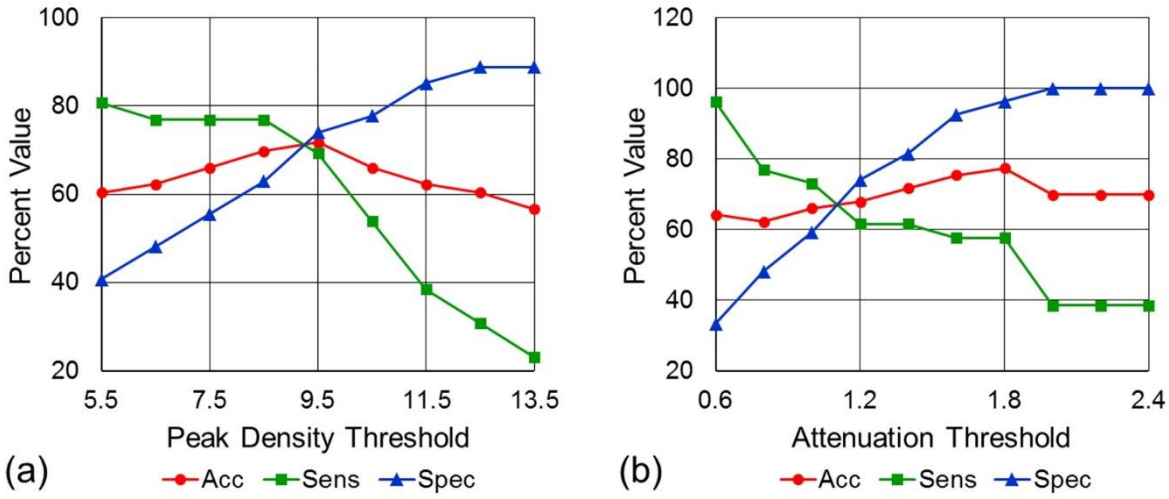

Figure 4: Threshold optimization for (a) peak density and (b) attenuation for the pilot study.

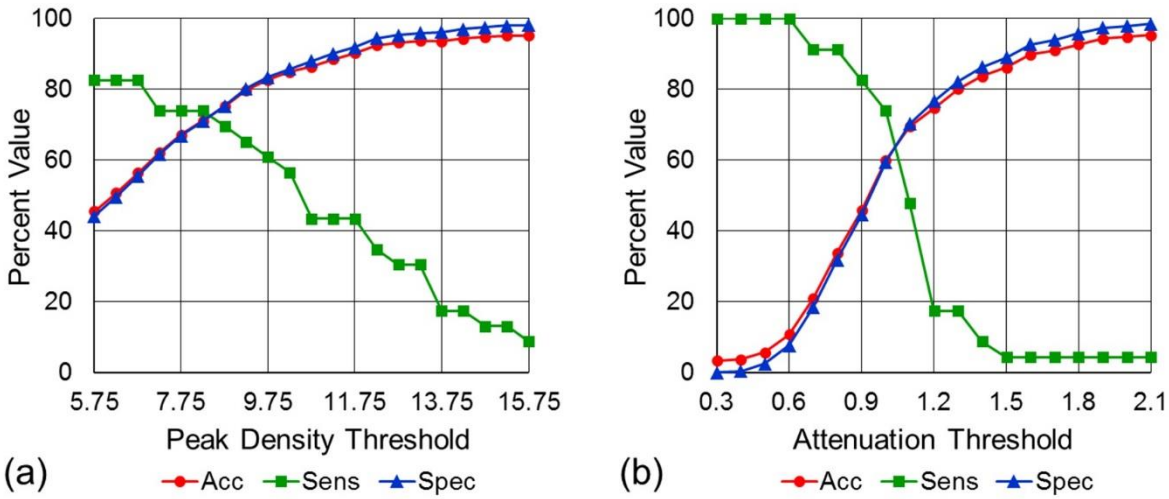

Figure 5: Threshold optimization for (a) peak density and (b) attenuation for the validation study.

The principal difference between the optimization curves for the pilot study (Figure 4) and the validation study (Figure 5) is the close trending of accuracy with specificity in the validation study. This is due to the very low number of malignant specimens tested (23) as compared to the number of nonmalignant specimens (326). In contrast, the pilot study comprised nearly equal numbers of malignant (26) and nonmalignant (27) specimens. Although very high specificities, and therefore accuracies, could be obtained for the validation study by setting the thresholds at high values, sensitivity drops dramatically and is sacrificed. Therefore, it was necessary to choose thresholds that provided similar values for all three statistical measures.

3.2 Comparison of Results

Figure 6 compares the statistical measures from the pilot and validation studies for the three data analysis methods: Multivariate (MV), peak density (PD), attenuation (AT). The results show that the validation study produced consistently higher sensitivities but lower specificities than the pilot study. This is most likely due to the method for selecting the thresholds for the validation study, which favored sensitivity, as compared to the pilot study, which favored accuracy and thus specificity. Attenuation shows the greatest variation in results between the pilot and validation studies. In contrast, peak density showed the least variation between the two studies, with all statistical measures remaining within a 5% range from 69.2% (pilot study sensitivity) to 74.1% (pilot study specificity). The results therefore indicate that peak density may be a more robust and consistent parameter than attenuation for the detection of malignant breast tissue in surgical margins.

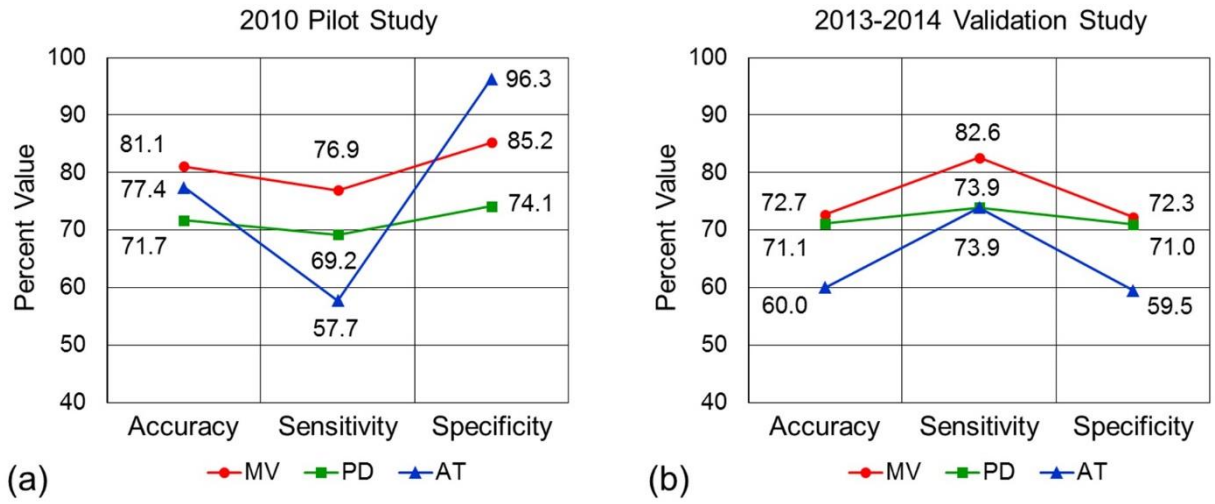

Figure 6: Comparison of results between pilot (a) and validation (b) studies.

The statistical measures from the HF ultrasound studies compare favorably with clinical trial results from two commercially available, noninvasive technologies for intraoperative BCS margin assessments [2,3]. The accuracy, sensitivity and specificity values for specimen radiography [2], and radio-frequency (RF) spectroscopy [3], were used as a benchmark for the multivariate results from the validation study (accuracy values for [2] and [3] were calculated from reported patient numbers). The results are shown in Figure 7, and demonstrate that HF ultrasound has comparable statistical measures to these alternative technologies.

There are two principal approaches to improving the statistical measures of the HF ultrasound method. First, the most significant source of error in the attenuation data was in the thickness measurements of the specimens during signal acquisition, which had an absolute error of ±0.5 mm and an average relative error of ± 9%. In contrast, studies with phantom specimens demonstrate that peak density is largely unaffected by specimen thickness. With greater precision in thickness measurements, such as obtainable with a motorized optical rail and encoder (1-µm resolution), the attenuation data could be substantially improved, thereby increasing their accuracy. Second, the sparse sampling of the specimens contributed substantially to statistical measures that were mostly below 90%. As shown in Figure 1(b), only

about 10-20% of each specimen was sampled by the HF ultrasonic measurements. The sampling area can be significantly increased with the implementation of an XY scanner with small-diameter transducers to automatically scan the surgical specimens and acquire data with higher resolution and from a much greater number of positions. Such a scanning approach would considerably improve the correlations between the HF ultrasound findings and pathology.

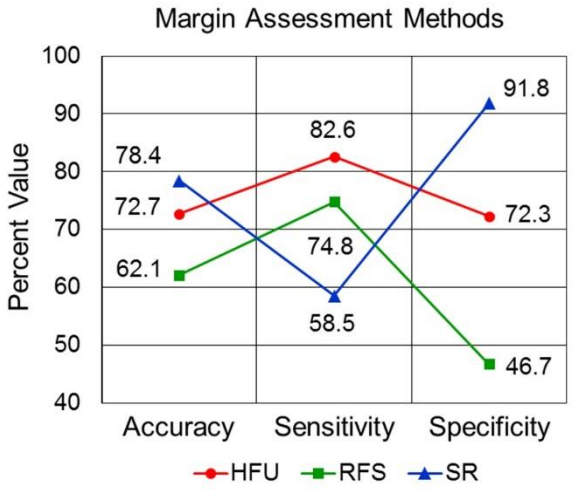

Figure 7: Comparison between high-frequency ultrasound (HFU), radio-frequency spectroscopy (RFS), and specimen radiography (SR).

4 Conclusions

The development of an intraoperative method to evaluate tissue margins is a central priority in breast conservation surgery. The goal of this research was to determine the accuracy, sensitivity, and specificity of high-frequency (20-80 MHz) ultrasound for detecting residual cancer in margins by conducting two studies—a 17-patient pilot study and 73-patient validation study—at the Huntsman Cancer Institute, Salt Lake City, Utah. Point measurements were acquired at 775 positions on 383 resected margin specimens using 50-MHz transducers in a through-transmission configuration. Attenuation and peak density were calculated from the ultrasonic waveforms and power spectra, respectively. Multivariate analyses combining the two parameters were also performed. The pilot and validation studies showed sensitivity values of 76.9% and 82.6%, respectively, and specificity values of 85.2% and 72.3%, respectively. The peak density results were particularly robust and consistent between the two studies, with all three statistical measures in the 69-74% range. The results also demonstrate that high-frequency ultrasound provides excellent statistical measures in comparison with RF spectroscopy and specimen radiography.

Acknowledgments

This research was funded by grants from the National Cancer Institute (NIH Award Number R21CA131798), the Elsa U. Pardee Foundation, the Eppley Foundation for Research, the Western Alliance for Expanding Student Opportunities (Arizona State University), the

Governor's Office of Economic Development of the State of Utah (Technology Commercialization and Innovation Program), the University of Utah, and Utah Valley University.

References

[1] Waljee, J.; Hu, E.; Newman, L.; Alderman, A. Predictors of re-excision among women undergoing breast-conserving surgery for cancer. *Annals of Surgical Oncology*, Vol 15 (5), 2008, pp 1297-1303.

[2] Bathla, L.; Harris, A.; Davey, M.; Sharma, P.; Silva, E. High resolution intra-operative two-dimensional specimen mammography and its impact on second operation for re-excision of positive margins at final pathology after breast conservation surgery. *American Journal of Surgery*, Vol 202 (4), 2011, pp 387-394.

[3] Schnabel, F.; Boolbol, S.; Gittleman, M.; Karni, T.; Tafra, L.; Feldman, S.; Police, A.; Friedman, N.; Karlan, S.; Holmes, D.; Willey, S.; Carmon, M.; Fernandez, K.; Akbari, S.; Harness, J.; Guerra, L.; Frazier, T.; Lane, K.; Simmons, R.; Estabrook, A.; Allweis, T. A randomized prospective study of lumpectomy margin assessment with use of MarginProbe in patients with nonpalpable breast malignancies. *Annals of Surgical Oncology*, Vol 21 (5), 2014, pp 1589-1595.

[4] Nguyen, F.; Zysk, A.; Chaney, E.; Kotynek, J.; Oliphant, U.; Bellafiore, F.; Rowland, K.; Johnson, P.; Boppart, S. Intraoperative evaluation of breast tumor margins with optical coherence tomography. *Cancer Research*, Vol 69 (22), 2009, pp 8790–8796.

[5] Haka, A.; Volynskaya, Z.; Gardecki, J.; Nazemi, J.; Shenk, R.; Wang, N.; Dasari, R.; Fitzmaurice, M.; Feld, M. Diagnosing breast cancer using Raman spectroscopy: prospective analysis. *Journal of Biomedical Optics*, Vol 14 (5), 2009, pp 054023.

[6] Doyle, T.; Factor, R.; Ellefson, C.; Sorensen, K.; Ambrose, B.; Goodrich, J.; Hart, V.; Jensen, S.; Patel, H.; Neumayer, L. High-frequency ultrasound for intraoperative margin assessments in breast conservation surgery: a feasibility study. *BMC Cancer*, Vol 11, 2011, pp 444.