Spectrophotometer and ultrasound evaluation of late toxicity following breast-cancer radiotherapy

E. J. Yoshida^{a)}, H. Chen, M. A. Torres, W. J. Curran, and T. Liu^{b)}

Department of Radiation Oncology, Emory University School of Medicine, Atlanta, Georgia 30322 and Winship Cancer Institute, Emory University, Atlanta, Georgia 30322

(Received 10 May 2011; revised 19 July 2011; accepted for publication 17 August 2011; published 27 September 2011)

Purpose: Radiation-induced normal-tissue toxicities are common, complex, and distressing side effects that affect 90% of patients receiving breast-cancer radiotherapy and 40% of patients post radiotherapy. In this study, the authors investigated the use of spectrophotometry and ultrasound to quantitatively measure radiation-induced skin discoloration and subcutaneous-tissue fibrosis. The study's purpose is to determine whether skin discoloration correlates with the development of fibrosis in breast-cancer radiotherapy.

Methods: Eighteen breast-cancer patients were enrolled in our initial study. All patients were previously treated with a standard course of radiation, and the median follow-up time was 22 months. The treated and untreated breasts were scanned with a spectrophotometer and an ultrasound. Two spectrophotometer parameters—melanin and erythema indices—were used to quantitatively assess skin discoloration. Two ultrasound parameters—skin thickness and Pearson coefficient of the hypodermis—were used to quantitatively assess severity of fibrosis. These measurements were correlated with clinical assessments (RTOG late morbidity scores).

Results: Significant measurement differences between the treated and contralateral breasts were observed among all patients: 27.3% mean increase in skin thickness ($p < 0.001$), 34.1% mean decrease in Pearson coefficient ($p < 0.001$), 27.3% mean increase in melanin ($p < 0.001$), and 22.6% mean increase in erythema ($p < 0.001$). All parameters except skin thickness correlated with RTOG scores. A moderate correlation exists between melanin and erythema; however, spectrophotometer parameters do not correlate with ultrasound parameters.

Conclusions: Spectrophotometry and quantitative ultrasound are objective tools that assess radiation-induced tissue injury. Spectrophotometer parameters did not correlate with those of quantitative ultrasound suggesting that skin discoloration cannot be used as a marker for subcutaneous fibrosis. These tools may prove useful for the reduction of radiation morbidities and improvement of patient quality of life. © 2011 American Association of Physicists in Medicine. [DOI: 10.1118/1.3633942]

Key words: radiation toxicity, breast cancer, spectrophotometer, ultrasound

INTRODUCTION

For the majority of women with breast cancer, radiation therapy is an essential component of treatment that reduces risk of local recurrence as well as improves breast preservation and survival. However, radiation-induced normal-tissue toxicities are common, complex, and distressing. Acute toxicity develops within days to weeks after treatment affecting over 90% of women who receive breast-cancer radiotherapy.¹ Late toxicity may occur months to years later affecting 30% to 40% of postirradiation patients.^{2–6} Although not as prevalent as acute, late toxicities are particularly concerning due to their persistent, and often permanent, manifestations.

Radiation Therapy Oncology Group (RTOG) established a late radiation morbidity scoring scheme and categorized late toxicities into two types: skin toxicity and subcutaneous toxicity (Table I).^{7–9} Based on clinical exam findings, physicians assigned a grade from 0 (absent) to 4 (severe). Skin toxicity is primarily evaluated by visual inspection based on changes in color (usually melanin), while subcutaneous-tissue toxicity is primarily evaluated by palpation. One serious problem of this grading scheme is the inherent sub-

jectivity of qualifying changes as “slight” (grade 1), “moderate” (grade 2), or “marked/severe” (grade 3). Furthermore, such assessments do not provide sufficient information to characterize the pathophysiology of tissue change.

Due to the limited capabilities of standard toxicity assessments, the natural history and biological mechanisms of radiation toxicities are still not completely understood. One question that remains is whether skin toxicity correlates with subcutaneous-tissue toxicity. To address this, we investigated two objective assessment tools: narrow band spectrophotometry and quantitative ultrasound.

The narrow band spectrophotometer measures skin injury through melanin and erythema indices.¹⁰ Pigmentation change is a common side effect of irradiation. Melanin and erythema indices are markers of skin discoloration. Melanin is the major pigment synthesized within membrane-bound melanosomes at the basal layer of the epidermis. Erythema describes redness of the skin resulting from the dilation of capillaries. Erythema may suggest the development of telangiectasias, which is another common radiation toxicity of the skin.

TABLE I. Radiation Therapy Oncology Group (RTOG) late radiation morbidity scoring scheme.

Organ/tissue	0	Grade 1	Grade 2	Grade 3	Grade 4
Skin	None	Slight atrophy, pigmentation changes, some hair loss	Patchy atrophy, moderate telangiectasia, total hair loss	Marked atrophy, gross telangiectasia	Ulceration
Subcutaneous tissue	None	Slight induration (fibrosis) and loss of subcutaneous fat	Moderate fibrosis but asymptomatic; slight field contracture; <10% linear reduction	Severe induration and loss of subcutaneous tissue; field contracture > 10% linear measurement	Necrosis

Note: Any toxicity that causes death is Grade 5.

Our quantitative ultrasound technique measures changes in subcutaneous injury through skin thickness and Pearson coefficient.^{11–14} Skin thickening is a well-known response to radiation that may be measured by the distance between the radio-frequency (RF) echo from the skin's surface and from that of the hypodermis. Radiation-induced damage to the basal layer of dermal cells results in a blurred and irregular dermal-hypodermal interface on B-mode ultrasound images. To this end, we calculated the Pearson correlation coefficient of envelop signals of the adjacent scan lines along the dermal-hypodermal interface in order to quantify its integrity. All measurements were validated through comparison with clinical assessment.

In summary, the purposes of this study were: (1) to investigate the combined use of spectrophotometry and quantitative ultrasound for radiation-induced late toxicity assessment; (2) to determine whether radiation-induced skin discoloration may be used as a marker for subcutaneous-tissue fibrosis in breast-cancer radiotherapy.

METHODS

Patient scanning

Eighteen breast cancer patients, who had previously received breast conservation therapy (lumpectomy and breast irradiation) for early-stage unilateral breast cancer, were enrolled in an Institutional Review Board approved study (Table II). Prior to enrollment, all patients had received a standard course of radiation: 50.0 to 50.4 Gy (1.8–2.0 Gy/fraction) delivered to the treated breast using a pair of parallel and opposed tangential 6-MV beams, followed by an electron boost of 10.0 to 16.0 Gy delivered to the lumpectomy site. The location of the prescribed dose was two-thirds of the perpendicular distance from the skin surface to the posterior border of the tangent field, at mid-separation on the central axis slice. Wedges were used to maintain the maximum dose within 7% of the prescription. Each patient received one quantitative imaging study during her regular follow-up visit. The time interval between treatment completion and follow-up scan ranged from 6 to 92 months (median of 22 months).

All patients received spectrophotometer and ultrasound studies, each requiring approximately 5 minutes. The patients were first scanned in supine position with spectrophotometer, Mexameter MX (Courage + Khazaka Electronic GmbH, Germany). Spectrophotometer scans were acquired

at the 12:00, 3:00, 6:00, and 9:00 positions on the treated and contralateral breasts (Fig. 1). Spectrophotometer measurements were repeated three times and averaged for greater reliability. The patients were then scanned with clinical ultrasound, Sonix RP (Ultrasonix Medical Corporation, BC, Canada). A linear array probe was employed to acquire B-mode images and RF echo signals at a 6-MHz center frequency. All ultrasound data were acquired using the same setting: 10 MHz frequency, 1.25 cm focal length, 4 cm depth, 72% gain, and 80 dB dynamic range. The scans were similarly taken at the 12:00 (upper), 3:00 (medial right breast/lateral left breast), 6:00 (lower), and 9:00 (lateral right breast/medial left breast) positions on the treated and contralateral breasts (Fig. 2, reproduction of a figure in Ref. 11). The spectrophotometer and ultrasound data were stored and analyzed offline.

Quantitative toxicity assessment

Mexameter MX measures melanin and erythema based on the diffuse remittance spectrometry principle. The spectrophotometer probe emits light of three wavelengths: 568 nm (green light), 660 nm (red light), and 880 nm (infrared light). Melanin index is calculated from the intensity of

TABLE II. Patient related characteristics.

Characteristics	Number
Age (year)	
Mean	56
Range	44 to 74
Follow-up time (month)	
Mean	22 months
Range	6 to 92 months
Race	
Asian	1 (6%)
African American	3 (17%)
Caucasians	5 (28%)
Hispanics	9 (50%)
Tumor stage	
Stage 0	3 (17%)
Stage I	11 (61%)
Stage II	4 (22%)
Tumor location	
Right breast	11 (61%)
Left breast	7 (39%)

FIG. 1. Diagram showing (a) the Mexameter MX with circular probe of 0.5 cm diameter and 0.2 cm² surface area, and (b) the location of Mexameter breast scans.

red light absorption and infrared light reflection, according to the equation

$$\text{Melanin index} = \log_{10} \left(\frac{\text{infrared signal}}{\text{Red signal}} \right) \quad (1)$$

Similarly, the erythema index is based on blood pigment absorption of green light and reflection of red light, related by the equation

$$\text{Erythema index} = \log_{10} \left(\frac{\text{Red signal}}{\text{Green signal}} \right) \quad (2)$$

Measurements from the upper, medial, lower, and lateral locations were averaged for each spectrophotometer parameter to reflect overall skin characteristics of the entire breast.

We have validated our quantitative ultrasound technology in the assessment of normal-tissue toxicity in a previous study.^{12,13} Ultrasound parameters were computed from RF data using in-house MATLAB software. Skin thickness was determined from the product of the ultrasound wave propagation speed in breast tissue, and the time interval between RF signal interaction between the epidermis and hypodermis. Skin thickness is represented by the expression

$$\text{Skin thickness} = \frac{vM}{2f_s}, \quad (3)$$

where v is the speed of sound, M is the sample points of the skin, and f_s is the ultrasound sampling frequency. The speed of sound in the human skin is 1640 m/s.¹⁵ In this study, the skin surfaces were contoured in all patients by one observer. We have demonstrated the reliability of inter-observer and intra-observer skin delineation in a previous report.¹³

The Pearson coefficient of the hypodermis, an average of the Pearson coefficient values of the envelop signals from adjacent acoustic scan lines, was calculated from RF signals within a 10 × 2 mm region-of-interest (ROI) situated along the hypodermal surface. The scan line density is 67.4 lines/cm. The Pearson coefficient was calculated using the following equation:

$$\text{Pearson coefficient} = \frac{\sum (s_i - \bar{s}_i)(s_j - \bar{s}_j)}{(m-1)\sigma_i\sigma_j} \quad (4)$$

where s_i and s_j are the Hilbert transforms of RF data from the i th and j th lines within the ROI, $\bar{s}_i$ and $\bar{s}_j$ are the sample means, σ_i and σ_j are the sample standard deviations, and m is the total number of lines in the ROI. Ultrasound analysis was performed without knowledge of spectrophotometer measurements or RTOG scores.

FIG. 2. Diagram showing (a) the orientation of the ultrasound probe, and (b) the locations of the breast ultrasound scans.

FIG. 3. (a) Spectrophotometer-measured melanin values of the untreated and treated breasts for all patients. (b) Melanin range and mean are shown for the untreated breasts and for the treated breasts stratified by RTOG late-toxicity score.

Clinical assessment

Clinical assessment was performed at the same time of ultrasound and spectrophotometer scans. One physician performed the physical examination, including inspection and palpation of both breasts. Each treated breast was compared to the contralateral breast for size, symmetry, contour, skin color, and texture. An RTOG late radiation morbidity scoring scheme was used for assessment of the treated breast skin (Table I). This scoring scheme is based on a subjective evaluation of the irradiated breast for which a grade 0 (no toxicity), 1 (mild toxicity), 2 (moderate toxicity), 3 (severe toxicity), or 4 (ulceration) is assigned based on the combined toxicity effects of atrophy, pigmentation change, and telangiectasia. Physicians were blinded to the quantitative assess-

ments of the ultrasound and spectrophotometer. The ultrasound and spectrophotometer analyses were performed without knowledge of clinical assessments.

Statistical analysis

Average parameter values obtained from the treated breast were compared to those of the untreated breast. A paired two-tail *t*-test was calculated for the four average parameter values from each breast. We evaluated and correlated melanin, erythema, skin thickness, and Pearson coefficient with respect to RTOG scores. Specifically, we sorted the parameter values into three groups: (1) parameter values of patients assigned an RTOG score of 0; (2) parameter values of patients assigned an RTOG score of 1; and (3)

FIG. 4. (a) Spectrophotometer-measured erythema values of the treated and untreated breasts for all patients. (b) Erythema range and mean are shown for the untreated breasts and for the treated breasts stratified by RTOG late-toxicity score.

parameter values of patients assigned an RTOG score of 2. No patient experienced severe toxicity (grade 3) or ulceration (grade 4).

RESULTS

Spectrophotometer measurements of skin discolor

Differences in melanin index value between the treated and untreated breasts were significant and correlated with RTOG skin late-toxicity grade. Of the 18 patients analyzed, 16 demonstrated an increase in melanin index of the treated breast, as shown in Fig. 3(a). The two patients with decreased melanin index did not experience radiation-induced side effects. The average melanin index of the

treated-breast skin was 289 (range: 9.4–810.5), while that of the untreated-breast skin was 227 (range: 28.1–775.1). A mean increase of 27.3% in melanin index ($p < 0.001$) of all patients was observed. Figure 3(b) shows an association between average increase in melanin index and RTOG skin-toxicity grade. Melanin index increased 2.6% for RTOG grade-0, 30.6% for grade-1, and 43.0% for grade-2 skin toxicity (Fig. 3(b)).

Significant differences in erythema index were observed between the treated and untreated breasts, which correlated with RTOG skin-toxicity grade. Fifteen of the 18 patients demonstrated an increase in erythema index of the treated breast, as shown in Fig. 4(a). The two patients with decreased erythema index did not experience radiation-

induced side effects. Average erythema index of the treated-breast skin was 448.0 (range: 100.0–455.8), while that of the untreated-breast skin was 304.0 (range: 114.3–495.3). A mean increase of 22.6% in erythema index ($p < 0.001$) was observed. Analysis of percentage changes in parameter values by RTOG grade revealed greater absolute percentage changes with increasing RTOG grade for erythema [Fig. 4(b)]. Erythema index increased 1.4% for RTOG grade-0, 28.3% for grade-1, and 30.8% for grade-2 late toxicity [Fig. 4(b)].

Ultrasound measurements of fibrosis

Significant skin thickening occurred in the treated breast when compared to the untreated breast (Table III); however,

TABLE III. Average spectrophotometer and ultrasound measures of treated and untreated breasts.

	Mean (standard deviation)		Difference	<i>p</i> -Value
	Untreated breast	Treated breast		
Melanin	227 (± 210)	289 (± 246)	62	<0.001
Erythema	248 (± 107)	304 (± 109)	56	<0.001
Skin thickness (mm)	2.05 (± 0.22)	2.61 (± 0.52)	0.56	<0.001
Pearson coefficient	0.41 (± 0.07)	0.28 (± 0.05)	-0.14	<0.001

skin thickness did not correlate with RTOG toxicity grade. Seventeen of the 18 patients presented with an increase in skin thickness of the treated breast [Fig. 5(a)]. Average

FIG. 5. (a) Ultrasound-measured skin thickness of the untreated and treated breasts for all patients. (b) Skin thickness range and mean are shown for the untreated breasts and for the treated breasts stratified by RTOG late-toxicity score.

thickness of the treated-breast skin was 2.61 mm (range: 1.53–3.65 mm), while that of the untreated-breast skin was 2.05 mm (range: 1.66–2.41 mm). A mean increase of 27.3% in skin thickness ($p < 0.001$) for all patients was observed [Fig. 5(b)]. Skin thickness increased by 38.4% for patients with RTOG grade-0, 23.8% for patients with grade-1, and 31.1% for patients with grade-2 toxicity.

The Pearson coefficient of the hypodermis significantly decreased in the treated breast when compared to the untreated breast (Table III), and the difference in Pearson coefficient correlated with RTOG grade. Seventeen of the 18 patients demonstrated a decrease in Pearson coefficient of the treated breast [Fig. 6(a)]. Average Pearson coefficient for the treated breast was 0.28 (range: 0.21–0.41), while that

of the untreated breast was 0.41 (range: 0.03–0.52). A mean decrease of 34.1% in Pearson coefficient ($p < 0.001$) was observed. The Pearson coefficient decreased by 18.4% for RTOG grade-0, 35.0% for grade-1, and 42.6% for grade-2 toxicity [Fig. 6(b)].

Correlation between skin toxicity and subcutaneous-tissue toxicity

Table IV lists the average percentage change in parameter values between the treated breasts and the untreated breasts, relative to RTOG score. A large variation in spectrophotometer measurements and a small variation in ultrasound measurements were observed among the contralateral breasts of

FIG. 6. (a) Ultrasound-measured Pearson coefficient along the hypodermal surfaces of the untreated and treated breasts for all patients. (b) The range and mean of Pearson coefficient for the hypodermal surfaces are shown for the untreated breasts and for the treated breasts stratified by RTOG late-toxicity score.

TABLE IV. Average percentage change of spectrophotometer and ultrasound measures by RTOG score.

	RTOG		
	0	1	2
Melanin	2.6%	30.6%	43.0%
Erythema	1.4%	28.3%	30.8%
Skin thickness (mm)	38.4%	23.8%	31.1%
Pearson coefficient	18.4%	35.0%	42.6%

the patients. To account for this baseline variation, we evaluated radiation toxicity level using relative parameter values, and subsequently examined cross-correlation between all parameters.

We computed the cross-correlation coefficients among the four parameters (Table V). A moderate correlation was observed between melanin and erythema. No correlation was observed between skin thickness and Pearson coefficient. No correlation was observed between spectrophotometer and ultrasound parameters (cross-correlation coefficients were less than 0.15).

DISCUSSION

In this clinical study, we compared spectrophotometry and quantitative ultrasound as assessment tools of late toxicity in breast-cancer radiotherapy. Two spectrophotometer parameters (melanin and erythema) were used to quantify changes in skin color. Two ultrasound parameters (skin thickness and Pearson coefficient) that measure structural changes of the parenchyma were used to evaluate subcutaneous fibrosis. We did not observe a correlation between ultrasound and spectrophotometer parameter values suggesting that skin discoloration does not correlate with the development of fibrosis.

The breast is composed of complex structures that change over time and vary significantly among individuals. Both baseline (contralateral) and irradiated breast characteristics among our patient population reflected this diversity (Table III). For example, spectrophotometer melanin measurements of fair-skinned women were generally within the range of 50-250, while those of dark-skinned women were generally within the range of 600-999. To account for these variations, we examined relative changes for each parameter—a comparison of the ratios of the treated to untreated breast. All four parameters demonstrated significant differences in the treated breast with respect to the contralateral breast (Table III). We validated these relative changes with RTOG clinical assessments (Table IV) and compared quantitative ultrasound with spectrophotometer parameters (Table V).

Of the five patients who had no signs of late toxicity after radiotherapy (RTOG = 0), 2 patients had a decrease in melanin index and two patients had a decrease in erythema index. Those who were found to have a decrease in postradiation melanin have very fair skin (melanin < 100) at baseline, while those who were found to have a decrease in post-radiation erythema have either very fair (melanin < 100) or very dark (melanin > 750) skin at baseline. This may suggest

TABLE V. Cross-correlation coefficient of spectrophotometer and ultrasound parameters.

	Melanin	Erythema	Skin thickness	Pearson coefficient
Melanin	—	0.6958	0.0356	−0.2872
Erythema	0.6958	—	0.1469	−0.0448
Skin thickness	0.0356	0.1469	—	0.0053
Pearson coefficient	−0.2872	−0.0448	0.0053	—

that a relative change in melanin index is a better marker of toxicity than erythema index in women with darker skin.

For this study, we used the average value of four breast quadrants for each of the four parameters to represent overall changes in the irradiated breast. One limitation of this approach is that the average parameter values do not differentiate the boosted (receiving doses of 60.0-66.4 Gy) from the nonboosted (receiving doses ≤ 50.0 Gy) regions. Further, the small number of patients enrolled and the absence of patients with high-grade tissue toxicity must be addressed in future studies. Despite the study's limitations, our preliminary results suggest that skin discoloration cannot be used as a marker for fibrosis. Moreover, these findings provide early evidence that skin toxicity and subcutaneous toxicity require separate objective evaluations.

A detailed understanding of nontargeted normal-tissue response is necessary for the optimization of radiation treatment in cancer therapy. Quantitative techniques of toxicity evaluation provide more specific measures than subjective RTOG scoring criteria, which are confined to 5 grades. This tool may be useful in clinical trials to compare various treatment strategies, such as external beam radiation vs MammoSite or standard dose fractionation vs hypofractionation. Although this study was conducted in breast-cancer radiotherapy, both spectrophotometry and quantitative ultrasound can be easily adapted for other treatment sites, such as the head and neck. These quantitative toxicity assessment tools will prove valuable as we continue to enhance our understanding and management of radiation toxicity in breast-cancer radiotherapy.

ACKNOWLEDGMENTS

This research was supported in part by National Cancer Institute Grant CA114313 and Susan Komen for the Cure foundation. A portion of this paper was presented at ASTRO 2009 and the authors have no conflict of interest.

^{a)}The first author is presently affiliated with the University of California Davis School of Medicine, and was supported in part by the Emory University Medical Scholars Award.

^{b)}Author to whom correspondence should be addressed. Electronic mail: tliu34@emory.edu; Telephone: (404)778-1848; Fax: (404)778-4139.

¹D. Porock and L. Kristjanson, "Skin reactions during radiotherapy for breast cancer: the use and impact of topical agents and dressings," *Eur. J. Cancer Care* **8**, 143-153 (1999).

²S. Collette, L. Collette, T. Budiharto, J. C. Horiot, P. M. Poortmans, H. Struikmans, W. Van den Bogaer, A. Fourquet, J. J. Jager, W. Hoogenraad, R. P. Mueller, J. Kurtz, D. A. Morgan, J. B. Dubois, E. Salamon, R. Mirimanoff, M. Bolla, M. Van der Hulst, C. C. Warlam-Rodenhuis, and H. Bartelink, "Predictors of the risk of fibrosis at 10 years after breast conserving therapy for early breast cancer: A study based on the EORTC Trial

- 22881-10882 boost versus no boost," *Eur. J. Cancer* **44**, 2587–2599 (2008).
- ³B. Emami, J. Lyman, A. Brown, L. Coia, M. Goitein, J. E. Munzenrider, B. Shank, L. J. Solin, and M. Wesson, "Tolerance of normal tissue to therapeutic irradiation," *Int. J. Radiat. Oncol. Biol. Phys.* **21**, 109–122 (1991).
- ⁴B. Fisher, S. Anderson, J. Bryant, R. G. Margolese, M. Deutsch, E. R. Fisher, J. H. Jeong, and N. Wolmark, "Twenty-year follow-up of a randomized trial comparing total mastectomy, lumpectomy, and lumpectomy plus irradiation for the treatment of invasive breast cancer," *N. Engl. J. Med.* **347**, 1233–1241 (2002).
- ⁵J. L. Harper, L. E. Franklin, J. M. Jenrette, and E. G. Aguero, "Skin toxicity during breast irradiation: pathophysiology and management," *South Med. J.* **97**, 989–993 (2004).
- ⁶R. R. Kuske, K. Winter, D. W. Arthur, J. Bolton, R. Rabinovitch, J. White, W. Hanson, and R. M. Wilenzick, "Phase II trial of brachytherapy alone after lumpectomy for select breast cancer: toxicity analysis of RTOG 95-17," *Int. J. Radiat. Oncol. Biol. Phys.* **65**, 45–51 (2006).
- ⁷S. M. Bentzen, W. Dorr, M. S. Anscher, J. W. Denham, M. Hauer-Jensen, L. B. Marks, and J. Williams, "Normal tissue effects: reporting and analysis," *Semin. Radiat. Oncol.* **13**, 189–202 (2003).
- ⁸J. D. Cox, J. Stetz, and T. F. Pajak, "Toxicity criteria of the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC)," *Int. J. Radiat. Oncol. Biol. Phys.* **31**, 1341–1346 (1995).
- ⁹F. Momm, S. Bartelt, K. Haigis, A. Grosse-Sender, and G. Witucki, "Spectrophotometric skin measurements correlate with EORTC/RTOG-common toxicity criteria," *Strahlenther. Onkol.* **181**, 392–395 (2005).
- ¹⁰Y. P. Huang, Y. P. Zheng, S. F. Leung, and A. F. Mak, "Reliability of measurement of skin ultrasonic properties in vivo: a potential technique for assessing irradiated skin," *Skin Res. Technol.* **13**, 55–61 (2007).
- ¹¹T. Liu, J. Zhou, K. S. Osterman, P. Zhang, S. A. Woodhouse, P. B. Schiff, and G. J. Kutcher, "Measurements of radiation-induced skin changes in breast-cancer radiation therapy using ultrasonic imaging," *BMEI 2008: Proceedings of the International Conference on Biomedical Engineering and Informatics*, Vol. **2**, 718–722 (2008).
- ¹²T. Liu, J. Zhou, E. J. Yoshida, S. A. Woodhouse, P. B. Schiff, T. J. Wang, Z. F. Lu, E. Pile-Spellman, P. Zhang, and G. J. Kutcher, "Quantitative ultrasonic evaluation of radiation-induced late tissue toxicity: pilot study of breast cancer radiotherapy," *Int. J. Radiat. Oncol. Biol. Phys.* **78**, 811–820 (2010).
- ¹³E. J. Yoshida, H. Chen, M. Torres, F. Andic, H. Y. Liu, Z. Chen, X. Sun, W. J. Curran, and T. Liu, "Reliability of quantitative ultrasonic assessment of normal-tissue toxicity in breast cancer radiotherapy," *J. Radiat. Oncol. Biol. Phys.* (in press), see [http://www.redjournal.org/article/S0360-3016\(10\)01559-2/fulltext](http://www.redjournal.org/article/S0360-3016(10)01559-2/fulltext) (accessed September 4, 2011).
- ¹⁴J. Zhou, P. Zhang, K. S. Osterman, S. A. Woodhouse, P. B. Schiff, E. J. Yoshida, Z. F. Lu, E. R. Pile-Spellman, G. J. Kutcher, and T. Liu, "Implementation and validation of an ultrasonic tissue characterization technique for quantitative assessment of normal-tissue toxicity in radiation therapy," *Med. Phys.* **36**, 1643–1650 (2009).
- ¹⁵C. M. Moran, N. L. Bush, and J. C. Bamber, "Ultrasonic propagation properties of excised human skin," *Ultrasound Med. Biol.* **21**, 1177–1190 (1995).