Computer aided prognosis for cell death categorization and prediction in vivo using quantitative ultrasound and machine learning techniques

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Purpose: At present, a one-size-fits-all approach is typically used for cancer therapy in patients. This is mainly because there is no current imaging-based clinical standard for the early assessment and monitoring of cancer treatment response. Here, the authors have developed, for the first time, a complete computer-aided-prognosis (CAP) system based on multiparametric quantitative ultrasound (QUS) spectroscopy methods in association with texture descriptors and advanced machine learning techniques. This system was used to noninvasively categorize and predict cell death levels in fibrosarcoma mouse tumors treated using ultrasound-stimulated microbubbles as novel endothelial-cell radiosensitizers.

Methods: Sarcoma xenograft tumor-bearing mice were treated using ultrasound-stimulated microbubbles, alone or in combination with x-ray radiation therapy, as a new antivascular treatment. Therapy effects were assessed at 2–3, 24, and 72 h after treatment using a high-frequency ultrasound. Two-dimensional spectral parametric maps were generated using the power spectra of the raw radiofrequency echo signal. Subsequently, the distances between “pretreatment” and “post-treatment” scans were computed as an indication of treatment efficacy, using a kernel-based metric on textural features extracted from 2D parametric maps. A supervised learning paradigm was used to either categorize cell death levels as low, medium, or high using a classifier, or to “continuously” predict the levels of cell death using a regressor.

Results: The developed CAP system performed at a high level for the classification of cell death levels. The area under curve of the receiver operating characteristic was 0.87 for the classification of cell death levels to both low/medium and medium/high levels. Moreover, the prediction of cell death levels using the proposed CAP system achieved a good correlation ($r = 0.68, p < 0.001$) with histological cell death levels as the ground truth. A statistical test of significance between individual treatment groups with the corresponding control group demonstrated that the predicted levels indicated the same significant changes in cell death as those indicated by the ground-truth levels.

Conclusions: The technology developed in this study addresses a gap in the current standard of care by introducing a quality control step that generates potentially actionable metrics needed to enhance treatment decision-making. The study establishes a noninvasive framework for quantifying levels of cancer treatment response developed preclinically in tumors using QUS imaging in conjunction with machine learning techniques. The framework can potentially facilitate the detection of refractory responses in patients to a certain cancer treatment early on in the course of therapy to enable switching to more efficacious treatments. © 2016 American Association of Physicists in Medicine.

Key words: cancer therapy, computer aided prognosis, kernel methods, microbubbles, quantitative ultrasound
1. INTRODUCTION

1.A. Quantitative ultrasound imaging

The availability of different cancer therapeutics, including chemotherapy, radiotherapy, targeted antibodies, and anti-vascular agents, is leading toward forms of more personalized cancer therapy that can optimize treatment efficacy while avoiding excessive and unnecessary toxicity. The shift from predetermined to individualized and adaptive treatment regimens based on tumor response can now be potentially facilitated by accurate, predictive, and early assessments provided by forms of medical imaging used for cancer response monitoring.1–3

Therapeutic cancer response assessment has been traditionally facilitated using functional imaging modalities, such as single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), positron emission tomography (PET), and optical imaging.1,4,5 These modalities can potentially provide noninvasive cancer response assessment at a gross level early after the start of treatment. However, there are two main drawbacks to such approaches: they are relatively expensive, and they require the injection of a contrast agent or a radioactive substance, which therefore makes them less appealing for longitudinal studies of cancer response assessment.

In contrast, techniques based on quantitative ultrasound (QUS) imaging4,6 provide an inexpensive, noninvasive, and rapid imaging framework in order to assess early tumor responses associated with changes occurring at the cellular level during the administration of treatment administration. Compared with the aforementioned imaging modalities, ultrasound has a major advantage in which it relies on endogenous contrast for the evaluation of treatment effectiveness. Imaging contrast is generated due to alterations in bioacoustic properties of tissue during the process of cell death itself.7–9

Programmed cell death causes many morphological changes in tumor cells, including nuclear condensation and fragmentation, cell swelling, and chromatin dissolution, which have been demonstrated to directly or indirectly affect tumor ultrasound backscatter characteristics.9–11 For example, an initial nucleus condensation and fragmentation associated with cell death increases ultrasound backscatter intensity. This also increases the randomness of fairly regular backscatter distributions of microvoids, which can produce a large backscatter signal. However, end-stage chromatin dissolution (nucleus degeneration) due to apoptotic nuclear degradation associated with necrosis reduces the amplitude of backscattered signal.12–14

In QUS, the raw radiofrequency (RF) data are used instead of ultrasound B-mode images, since the latter are image-processed and log-compressed datasets that are unreliable in depicting all frequency-dependent microstructure changes in the tumor during cell death.5 QUS methods are used to analyze the entire frequency-dependent power spectrum, capturing the effects of scattering microstructures more effectively than B-mode intensity images. QUS has advantages over conventional ultrasound (B-mode) imaging, as it uses metrics that are predominantly independent of the instrument settings, and is derived from the spectrum analysis (spectroscopy) of the backscatter RF signals over a region of interest, which can also be used to form parametric maps.

The applicability of QUS methods to detect different modalities of cell death, including mitotic arrest and apoptosis, has been demonstrated in several in vitro, in situ, and in vivo studies with multiple tumor types and using different therapeutic modalities.4,6,8,12,15–18 The sensitivity of high-frequency-ultrasound (20–50 MHz) backscatter intensity to cell death resulting from cancer treatment was initially demonstrated by Czarnota et al. in vitro8 and then in vivo.12 The research was extensively followed by in vitro and in vivo studies demonstrating the capability of high-frequency QUS methods in detecting and monitoring cell death occurring as a result of cancer treatment.9,17,19,20 In this study, high-frequency QUS was used in conjunction with state-of-the-art textural methods and advanced machine learning techniques for the assessment of ultrasound-stimulated microbubble enhancement of radiation response in fibrosarcoma xenograft tumor models.

1.B. Ultrasound stimulated microbubbles enhancement of radiotherapy

Radiotherapy is one of the leading cancer treatment modalities. It induces cell death primarily by DNA double strand break, which is a direct result of DNA ionization or an indirect effect of free radical generation.21,22 Due to its prevalence in cancer therapy, many attempts have been made to enhance the radiation therapeutic index.23 All these attempts have a common goal, which is the radiosensitizing of cancerous cells. In addition to various pharmaceutical and biochemical techniques that can lead to toxic effects,24 ultrasound-stimulated microbubbles have recently been proposed as a new treatment to enhance radiotherapy efficacy.25–27 This line of research is based on recent studies that have suggested tumor endothelial cells respond preferentially to single high doses of radiation via a ceramide-dependent mechanism,28–31 which may result in vascular destruction and, therefore, a secondary tumor cell death.29,32 The ceramide-dependent phenomenon in vascular responses is triggered by an upregulation of acid-sphingomyelinase (asmase) in the membrane of cells.32–36 Researchers have used the secondary cell death as a route to the enhancement of radiation response. For example, conventional vascular targeting agents, such as antiangiogenics, have been used to radiosensitize tumor cells prior to radiation therapy.37–39 More recently, ultrasound-mediated microbubbles have been demonstrated to act as a novel biophysical vascular perturbation agent, which also activate ceramide-dependent vascular collapse and tumor cell death.25–27,40–42

Although previous in vitro and in vivo studies demonstrated the effectiveness of ultrasound-mediated microbubbles in enhancing radiation therapy,25–27,41,42 they also placed more emphasis upon the biological attributes of the process. In order to complement this, here, steps are taken toward the design of a computer-aided-prognosis (CAP) system to noninvasively categorize the extent of cell death to low/medium/high levels (discretely) and also predict the percentage of cell death (continuously), which is important in providing an early
assessment of treatment effectiveness. To this end, for the first time, we propose a CAP system by using state-of-the-art textural methods and advanced machine learning techniques, in conjunction with high frequency QUS spectral methods in a supervised learning paradigm, to categorize and predict the level of cell death in wild type murine fibrosarcoma tumors treated by combined ultrasound-stimulated microbubbles and radiotherapy.

1.C. Textural methods and machine learning techniques

Due to the heterogeneous responses often developed in tumors as a result of cancer treatment, QUS parametric maps can be characterized using texture methods for a more accurate evaluation. Texture analysis has previously been adapted for tissue characterization in US conventional B-mode images and, more recently, for cancer response monitoring in QUS methods. Text analysis methods are generally classified into four groups of approaches: statistical, structural, transform-based, and model-based. As a complicated phenomenon, no unique definition has been provided for textures in the literature. Therefore, various texture methods have been proposed, each of which tries to model one or a few properties of texture. Among the existing methods, local binary patterns (LBPs), which are predefined binary operators, have led to state-of-the-art results in texture analysis. One main advantage of the LBPs is providing a unified statistical and structural approach for texture analysis.

After feature extraction, one major requirement in the design of a CAP system is to measure the distance between the “pretreatment” and “post-treatment” scan samples as an indication of treatment effectiveness. In the research presented here, a recently introduced kernel-based metric called maximum mean discrepancy (MMD) was adapted for this measuring purpose.

Maximum mean discrepancy is an advanced dissimilarity measure, particularly suitable for comparing two populations represented by multiple data instances. As a kernel-based measure, its computation is reliant on inner products taken in a reproducing kernel Hilbert space (RKHS). By using a kernel function to nonlinearly transform input vectors into a different, possibly higher-dimensional feature space, and by computing the population means in this new space, enhanced group separability (compared to Euclidean distance in the original feature space, for example) is ideally obtained.

1.D. Contributions

Previous studies on the applications of QUS methods in cell death detection were mainly limited to simple analyzies, such as statistical tests of significance and linear discriminant analyzes. These studies used restricted settings, such as the same training and test sets to demonstrate the proof of principle of the proposed QUS methods. However, state-of-the-art texture methods, advanced machine learning techniques, and supervised learning in a realistic setting (such as leave-one-subject-out validation scheme), as presented in this study, are needed in order to design a complete computer-aided-prognosis system for the early prediction (within hours after the start of treatment) of cell death levels reliably, and automatically, both at discrete and continuous levels. As a summary, the main contributions of this study are as follows:

1. The design and development of a complete, noninvasive CAP system based on QUS spectral methods, state-of-the-art texture analysis, a kernel-based dissimilarity measure, and a supervised learning paradigm in leave-one-subject-out validation scheme, in order to early predict cell death levels at discrete and continuous levels.

2. Applying the proposed CAP system (block diagram shown in Fig. 1) to detect early cell death due to radiation therapy in the context of a novel use of ultrasound-mediated microbubbles as radiosensitizing antivascular agents in a murine tumor line.

3. Providing one of the first studies to apply machine learning techniques, such as kernel-based methods, to QUS imaging of the effects of combined microbubbles and radiation therapy.

4. Quantifying the levels of cell death as low, medium, or high, noninvasively, with high accuracy, sensitivity, and specificity. In addition, successfully quantifying the continuous levels of cell death, which closely followed the ground-truth levels of cell death in all 27 treatment groups.

2. METHODS

2.A. Cell culture and animal preparation

Cancerous cells (MCA/129 fibrosarcoma) were cultured in DMEM essential medium (Wisent Inc., Montreal, QC, Canada), supplemented with 10% characterized fetal bovine serum (HyClone, Logan, UT, USA) and 1% penicillin-streptomycin (Gibco, Grand Island, NY, USA), and incubated at 37 °C under 5% CO₂. Cells were subsequently trypsinized (Gibco, Grand Island, NY, USA), pooled, and counted. Cell pellets were isolated and suspended in 50 µL of phosphate-buffered saline (PBS) per 1.0 × 10⁶ cells in preparation for inducing tumors in mice.

In this study, experiments were carried out using (n = 108) SV129/C57BL/6 asmase⁺⁺ mice. The prepared cancerous cells were injected subcutaneously to the right hind leg of each animal and tumors were permitted to grow to an average size of 1.0 cm for approximately 10 days.

Mice were anesthetized prior to imaging and treatment using a mixture of ketamine (100 mg/kg), xylazine (5 mg/kg), and acepromazine (1 mg/kg) administered via intraperitoneal (IP) injection. Animals were kept warm during the time they were asleep using heat lamps and/or heat pads. All animal experiments in this study were conducted in compliance with the guidelines specified by the Sunnybrook Health Sciences Centre Institutional Animal Care and Use Committee.
2.B. Treatment

Animals were administered one of nine possible therapeutic conditions, including: no treatment, ultrasound-stimulated microbubbles as radio-sensitizer (at low or high dose), radiotherapy alone (2 Gy or 8 Gy in single doses), or a permuted combination therapy consisting of ultrasound stimulated microbubbles followed by radiation therapy.25

2.B.1. Microbubbles and ultrasound-stimulated therapy

Definity microbubbles (Lantheus Medical Imaging Inc., N. Billerica, MA, USA) were used in ultrasound-stimulated treatment. The microbubbles were activated using a Vialmix mechanical shaking device (Lantheus Medical Imaging Inc.) for 45 s at 3000 rpm. Mice were mounted onto an acrylic jig with their hind legs submerged into a 37°C water bath, while their head remained dry. Low (1% v/v) and high (3% v/v) microbubble concentrations were calculated according to total animal blood volume estimated by its weight. The microbubbles were diluted in sterile normal saline, and a total of 100 µL was intravenously injected into the tail vein at a constant rate. This was followed by a secondary injection of 150 µL normal saline with 0.2% heparin to flush the tail vein before treatment onset. The microbubble injection was followed by an immediate ultrasound therapy.

The ultrasound-stimulated microbubble treatment was administered using the setup previously described by Lee et al.51 The setup consisted of a waveform generator (AWG 5014, Tektronix, Beavetron, OR, USA), a power amplifier (AR KAA4030P, AR Worldwide-Nodular RF, Bothell, WA, USA), a 500 kHz immersion transducer (IL 0509HP, Valpey Fisher Inc., Hopkinton, MA, USA), and a digital acquisition system (Acqiris DC440/PXI8570, Agilent Technologies, Mississauga, ON, Canada). The transducer had a diameter of 2.87 cm, focal depth of 8.5 cm, a peak negative pressure of 570 kPa, a −6 dB beamwidth of 31 mm, and a depth of field greater than 2 cm. The digital acquisition system was used to adjust the focal point of the ultrasound beam prior to experiments. A reference needle was placed in front of the transducer and monitored by the system so that the maximal signal was received at the focus. Treatment was performed underwater with the transducer located inside a water tank. The mouse was secured partially submerged in a 37°C water tank in a standing position, with its head and tail up and hind legs down. The tumor-bearing leg was oriented directly in front of the transducer at the distance calibrated for the maximal focused acoustic signal (8.5 cm). Immediately after the microbubble injection, the animals were exposed for 5 min of ultrasound treatment. Tumors were exposed to 16 cycles of a 500 kHz tone burst (yielding a total duration of $16 \times 2 \mu s = 32 \mu s$) followed by a gap, resulting in a pulse repetition frequency of 3 kHz, which was equivalent to a period of 333 µs. This pulse sequence was repeated for 150 periods within a 50 ms window, amounting to approximately 5 ms of total ultrasound exposure.59 The 50 ms window occurred every 2 s to permit blood vessels to refill with bubbles. For the total treatment time of 5 min (300 s), the ultrasound exposure...
time was 750 ms (150 periods × 5 ms), resulting in an average duty cycle of 0.25%.

2.B.2. Radiation therapy

Animals receiving radiation were administered X-radiation alone or immediately after microbubble therapy. Single fraction doses of 2 Gy or 8 Gy were administered using a Faxitron cabinet irradiator (Faxitron Biopics, Lincolnshire, IL, USA) at an energy of 160 kVp, a dose rate of 200 cGy/min, and source-skin distance of 30 cm. Animals were covered with a 3 mm thick lead shielding with an aperture to expose only the tumor.

2.B.3. Treatment groups

Treatment responses were assessed at 2/3, 24, or 72 h after treatment onset. Considering the nine aforementioned therapeutic conditions at the beginning of this subsection, there were 27 groups with four mice per group, resulting in 108 mice in total.

2.C. Ultrasound imaging

Ultrasound data were acquired from the whole tumor area in each animal immediately prior to treatment administration (pretreatment imaging), and after group-specified exposure time (post-treatment imaging). A high-frequency ultrasound imaging device (Visualsonics, Toronto, ON, Canada) with a single-element 30 MHz transducer (RMV-707B: 55 μm axial resolution, 115 μm lateral resolution, center frequency of 25 MHz, and focused at a 12.7 mm depth) was used for data acquisition. The device was used to collect 3D data with a scan plane separation of 0.2 mm (in hip-to-toe direction), and RF data were acquired with a sampling frequency of 500 MHz.

2.D. Histopathology

In order to obtain a ground truth for the extent of cell death, histological analysis was performed on excised tumor. To this end, animals were killed immediately after post-treatment imaging. Excised tumors were fixed in formalin, stored up to two days at room temperature, and then moved to a 90% ethanol solution and stored at 4°C for five additional days. From each tumor, three representative 5 μm thick paraffin-embedded sections were stained using hematoxylin and eosin (H&E), which was used for a general morphological survey of embedded sections were stained using hematoxylin and eosin (H&E), which was used for a general morphological survey of tumor alterations following therapy. Furthermore, in situ nick labeling (ISEL) immunohistochemistry was performed to quantify the level of cell death by computing the ratio of ISEL stained areas to whole tumor cross section area. This ground truth is referred to as the “histological cell death” levels/fraction/values in this study.

2.E. Quantitative ultrasound spectral analysis

Quantitative ultrasound spectral methods were used to obtain an estimate of the RF data power spectrum. A region of interest (ROI) was selected for further analysis from tumor centers. The ROIs were consistently positioned at the transducer focal depth and within the transducer’s depth of field. The ROI selection was performed across 20 scan planes on each animal, consisting of ten scans from pretreatment and another ten from post-treatment. By using a sliding window within the ROI on a pixel-by-pixel basis (i.e., shifting the sliding window one pixel at a time row-wise or column-wise), each selected ROI was segmented into smaller square blocks called RF blocks, consisting of several adjacent Hamming-gated RF segments, approximately equivalent to ten wavelengths or a time-bandwidth product of about seven. The power spectrum over each RF block was subsequently estimated by first computing the fast Fourier transform (FFT) of the Hamming-gated RF segments, then averaging it over each RF block. In order to remove the effects of system transfer function, the power spectrum of each window was normalized with a calibration pulse obtained from a flat quartz plate. The perpendicular reflection off the flat quartz plate located at the focal point of the transducer was used for the purpose of this normalization. The tumor ROIs were always selected within the depth of field of the transducer. Coaxial attenuators from the HAT Mini-Circuits™ series were used in order to avoid signal saturation when the quartz reflector was imaged. Any artificial attenuation of this kind was then accounted for in later calculations. Moreover, the normalized power spectra compensated for frequency-dependent attenuation in the tumor tissue. To this end, an attenuation coefficient of 0.06 dB/mm/MHz was considered for the tumor tissue. This value was computed in the homogeneous regions of the mouse tumors before and after treatment by measuring the linear rate of decrease in the midband fit (MBF) with increasing depth.

Linear regression analysis was performed on normalized power spectra, within a −6 dB bandwidth from the transducer’s center frequency determined from a calibration pulse (13.5-27.5 MHz), in order to generate a best-fit line. This analysis resulted in three spectral parametric maps, including: (a) MBF, which is the normalized power (dB) at the center frequency; (b) 0-MHz intercept or spectral intercept (SI), the intercept of the fitted line to the calibrated y-axis; and (c) spectral slope (SS), the slope of the fitted line. These spectral parameters are related to ultrasound backscatter power, acoustic concentration, and scatterer size, respectively.

The computation of these three parameters at the center pixel of the sliding window formed three parametric maps. Representative pretreatment and post-treatment MBF parametric maps overlaid on the B-mode images are presented in Fig. 2.

Different sets of QUS parameters have been applied in previous studies on tissue characterization and therapy response monitoring. In this study, MBF, SI, and SS were extracted. However, since MBF and SI provided better results in the classification and prediction of cell death, results were only reported for these two parametric maps.

2.F. Textural features-local binary patterns

Two-dimensional parametric maps were generated as a result of employing QUS spectral methods. In order to provide a more concise and discriminative/representative description of 2D maps, feature extraction techniques were utilized.
Treatment administration often results in developing heterogeneous responses in tumors. Therefore, textural features have recently been adapted to characterize the spectral parametric maps. Among texture methods in the literature, LBPs are considered to be one of the state-of-the-art techniques, which are based on some predefined binary operators. They were originally introduced by Ojala et al. as unified structural and statistical texture descriptors.

The LBPs were defined in a circular neighborhood of radius $R$ with $S$ evenly spaced surrounding pixels $x_s$ around a central pixel $x_c$ using

$$LBP_{S,R} = \sum_{x_s=1}^{S} H(I(x_s) - I(x_c)) 2^{s-1},$$

where $I(x)$ was the intensity at the pixel $x$, and $H(.)$ was the Heaviside step function. By considering the sign of differences — implemented using the Heaviside unit function $H$ in (1) — instead of the exact difference values $(I(x_s) - I(x_c))$, as long as the relative ordering between the gray scale values of the surrounding pixels and the center pixel does not change, the output of (1) remains unchanged, resulting in gray scale invariance, where gray scales are, for example, the values of pixels of false-colored QUS parametric maps. In addition, rotation invariant LBPs were used here by finding the lowest possible binary number obtained from rotating the circular operator defined in (1) as follows:

$$LBP_{S,R}^i = \min_{i=1,\ldots,S} \{ \text{ROR}(LBP_{S,R}^i) \},$$

where $\text{ROR}(x,i)$ was an $i$-bit right-rotation function on a $S$-bit number $x$.

As shown by Ojala et al., uniform patterns, i.e., the LBPs with minimal spatial transitions, are the fundamental properties of texture. This means that “uniform” patterns provide a vast majority of the $3 \times 3$ texture patterns in examined surface textures, sometimes accounting for more than 90% of these patterns. Therefore, uniform two rotation invariant LBPs (denoted as $\text{LBP}_{S,R}^{\text{ui2}}$) were used in this study, which were defined as follows:

$$LBP_{S,R}^{\text{ui2}} = \begin{cases} \sum_{x_s=1}^{S} H(I(x_s) - I(x_c)) & \text{if } U(LBP_{S,R}) \leq 2, \\ S + 1 & \text{otherwise}. \end{cases}$$

The function $U(.)$ in (3) returned the number of spatial transitions of a pattern as its argument. The $LBP_{S,R}$ is a $S$-bit binary operator (number) and each bit can take either 0 or 1 depending on the relative gray scale values of the corresponding surrounding pixel $x_s$ and center pixel $x_c$. The number of spatial transitions depends how many $0 \rightarrow 1$ and $1 \rightarrow 0$ bit transitions occur in $LBP_{S,R}$. For example, all the nine uniform patterns with maximum two spatial transitions are shown in Fig. 3, and the corresponding $LBP_{S,R}^{\text{ui2}}$ codes obtained using (3) are shown inside these patterns. As Fig. 3
shows, the uniform patterns are primitive texture elements such as spots, corners, edges, and smooth areas. In the context of cancer response monitoring, the prevalence of the primitive elements such as dots, corners, and edges in a parametric map indicates a more heterogeneous texture, which is speculated to correspond with more cell death. Availability of more smooth areas, on the other hand, indicates no cell death, corresponding with more homogeneous textures. This property of the LBPs was used in this study to discriminate different levels of cell death indicated in parametric maps.

According to its definition, the operator $LBP^{\text{uni}}_{S,R}$ generated $S+2$ distinct values. Thus, the corresponding histogram was of the same number of bins. A multiresolution realization of LBPs was achieved by using different pair values of $(S,R)$ without any blurring effect, as is common when employing filtering approaches. As recommended in Ref. 50, the pair values (8,1), (16,2), and (24,3) were used in this study.

2.G. Kernel-based metric

Cancer response monitoring requires a dissimilarity measure between the data samples taken from pretreatment and post-treatment as an indication of treatment effectiveness. In other words, after computation of features, e.g., LBP features described above, and for the purpose of therapeutic cancer response assessment, a large distance between the underlying distributions represented by the data samples from pretreatment and post-treatment indicates a successful treatment; if the distance between the distributions is small, the treatment is considered unsuccessful. This judgment is based on the previous work on QUS methods that demonstrated different modalities of cell death, such as apoptosis and mitotic arrest, change the bioacoustic properties of tissue reflected in spectral parametric maps.6,9,12

Perhaps the simplest and most straightforward way to measure the distance between two distributions is to calculate the distance between the cluster means using

$$d(p,q) = \|E(p) - E(q)\|_2^2,$$

where $E$ is the expectation function, $p$ and $q$ are the two distributions. The main drawback of the metric given in (4), which is equivalent to Euclidean distance or $\ell_2$ norm, is that it only takes into account the first order statistics of the data samples taken from $p$ and $q$. Therefore, if the two distributions have the same mean values, they cannot be discriminated using (4) even if, for example, their standard deviations (second order statistics) are different.

One approach to overcome this problem is to first map the data to a higher dimensional feature space, and then compute (4) in the augmented feature space (see Fig. 4 as an illustration). By computing the expectation function in the augmented feature space, higher order statistics of the two distributions are effectively taken into account, resulting in an enhanced discrimination. This idea was effectively and efficiently implemented by Gretton et al.,52,53,55 and led to a nonparametric (i.e., making no assumption on the distributions $p$ and $q$) kernel-based metric in RKHSs called MMD.

To provide a formal description, let $X = \{x_i\}_{i=1}^m$ and $Y = \{y_i\}_{i=1}^m$ be data samples drawn independently and identically distributed (i.i.d.) from $p$ and $q$, respectively. A feature mapping function $\varphi$ can be defined such that $X \sim p, X \xrightarrow{\varphi} \varphi(X)$ and similarly $Y \sim q, Y \xrightarrow{\varphi} \varphi(Y)$, which maps the data to a high dimensional feature space. By computing (4) in this space, a metric is computed with the following formulation:

$$\text{MMD}(\varphi,p,q) = \|E(\varphi(p)) - E(\varphi(q))\|_2^2$$

$$= \left[ E[(\varphi(X) - \varphi(Y))^\top((\varphi(X) - \varphi(Y))] \right]^{\frac{1}{2}}$$

$$= \left[ E[\varphi(X)^\top\varphi(X) - 2\varphi(X)^\top\varphi(Y) + \varphi(Y)^\top\varphi(Y)] \right]^{\frac{1}{2}}.$$ (5)

In practice, to compute (5) using a finite number of data samples $X = \{x_i\}_{i=1}^m$ and $Y = \{y_i\}_{i=1}^m$ taken from the two distributions $p$ and $q$, respectively, the following empirical formulation for MMD can be used:

$$\text{MMD}(\varphi,X,Y) = \frac{1}{m} \sum_{i,j} k(x_i,x_j) - \frac{2}{nm} \sum_{i,j} k(x_i,y_j)$$

$$+ \frac{1}{m^2} \sum_{i,j} k(y_i,y_j)^2,$$ (6)

where $k(x_i,x_j) = \langle \varphi(x_i),\varphi(x_j) \rangle$.

Fig. 4. Two Gaussian distributions with the same mean values and different variances. Since the mean of the two distributions are the same, using (4) by itself is not sufficient to discriminate them. However, by using a mapping such as $X \rightarrow [X X^2]$, the data are mapped to a higher dimensional feature space. By the computation of (4) in this augmented feature space, in fact, the second order statistic of the distributions (variance) is also taken into account. This, for example, can discriminate the two distributions.
Intuitively, the empirical MMD is expected to be small when \( p = q \) and large when the two distributions are far apart. It can be computed in quadratic time: for \( n + m \) data samples, the cost of computation is \( O((n+m)^2) \) time,\(^\text{72} \) which is reasonably low for a real-time computation. Empirical MMD was adapted in this study to compute the dissimilarity between the pretreatment and post-treatment samples of each animal.

### 2.H. Proposed computer-aided-prognosis system

The final step for the therapeutic cancer response assessment was to categorize/predict the level of cell death. This was accomplished by employing classifiers/regressors in a supervised learning paradigm on the computed dissimilarities between pretreatment and post-treatment scans.

To this end, in the proposed CAP system, after computing the histogram of intensities or LBP textural features on spectral parametric maps, resulting in a \( B \)-bin histogram, the MMD values were computed for each animal using \(^\text{76} \). A histogram intensity kernel (HIK) was used in \(^\text{6} \), which can be computed using

\[
  k_{\text{HIK}}(h_1, h_2) = \sum_{b=1}^{B} \min(h_{1b}, h_{2b}),
\]

where \( h_1 \) and \( h_2 \) are the two histograms with \( B \) bins and \( h_{1b} \) and \( h_{2b} \) values in each bin, respectively. HIK is a parameter-free kernel, which has been particularly recommended as the kernel of choice on histogram descriptors in other kernel-based methods.\(^\text{73,74} \)

The assessment of cancer response was performed in two ways in this study: first, categorizing of cell death to less/more than 20% or 40% using a binary classifier. Second, predicting the level of cell death using a regressor. For the purpose of classification, a naïve Bayes classifier was employed with the MMD values as dissimilarity features, and thresholded ground-truth cell death values to less/more than 20% (or 40%) as labels. This categorized the level of cell death to three levels: low (less than 20%), medium (between 20% and 40%), and high (more than 40%) as an indication of treatment effectiveness. The performance of the proposed CAP system in categorizing cell death levels as “low, medium, or high” was evaluated using several different measures including accuracy, area under curve (AUC) of the receiver operating characteristic (ROC), sensitivity, and specificity. On the other hand, in the assessment by cell death prediction, a linear support vector regressor (SVR) was employed with MMD values as predictor variables and ground-truth continuous cell death values as responses. The ultimate goal was to predict the level of cell death for each animal after training the regressor on a training set, which was performed in a leave-one-subject-out setting. The performance of the proposed CAP system in predicting continuous cell death levels was evaluated using Pearson’s correlation coefficient in comparison to ground-truth tumor cell death values obtained from histological analysis. The two methods provided complementary information to better assess the therapeutic response: one by providing hard-thresholded labels to low/medium/high level of cell death, and the other by providing a continuous value as the predicted cell death level.

### 3. RESULTS

#### 3.A. Alternative features and dissimilarity measures

In the proposed CAP system, textural features were computed using LBP method and the dissimilarity between the data samples taken from pretreatment and post-treatment were computed using the MMD with an HIK. The performance of the proposed CAP system using this feature–distance combination was compared with several other features and dissimilarities. In the previous studies on cancer response monitoring, gray level co-occurrence matrices (GLCMs)\(^\text{78} \) have recently been used to extract textural features from parametric maps.\(^\text{57,76} \) Therefore, a comparison was provided between the LBP and GLCM as a classical texture method. GLCM is considered to be a statistical texture analysis method, which is based on the joint probability of gray scales for the pair of pixels at a certain relative distance \( d \) and orientation \( \theta \). Second-order statistics, including contrast, correlation, energy, and homogeneity, were computed on the GLCM as texture features to represent each parametric map.

LBP is, by design, a gray-scale invariant texture method, meaning that the LBP texture descriptor is invariant to the local intensity level changes in a texture image. Therefore, in order to account for the changes in the parametric map intensity levels, which convey useful information in cancer response monitoring,\(^\text{77} \) the histogram of intensity (“HistInt”), and also the combination of LBP features and HistInt were considered in these comparisons. In the latter case, the concatenation of the normalized histograms computed over the intensity levels and LBP operators was considered as the feature set.

In order to demonstrate the contribution of a kernel-based metric, such as MMD, toward the overall performance of the proposed CAP system, a comparison was also provided with a nonkernel dissimilarity measure such as \( \ell_2 \) norm.

#### 3.B. Implementation details

Spectral parametric maps including MBF, SI, and SS were used in the experiments. However, since MBF and SI consistently outperformed SS, and since out of the three spectral parameters, only two of them are independent, the results were only reported on MBF and SI.

Rotation-invariant uniform two local binary patterns (LBP\(^\text{unl} <_{S,R} > \)) were used in this study at three different \((S,R)\) pair values: \((8,1)\), \((16,2)\), and \((24,3)\). These values realized a multiresolution implementation of the LBP. The histograms generated as the models/features for the parametric maps were of \( S + 2 \) bins: 10, 18, and 26 at the three different resolutions, respectively. One set of MMD values were computed at each resolution between the pretreatment and post-treatment scans, resulting in three sets of MMD values. A feature selection using sequential forward selection (SFS) algorithm in a wrapper framework\(^\text{78} \) was used on the training set to find the best resolution.

The computation of GLCM was performed over a combination of ten distances from 1 to 10 pixels and four orientations at 0°, 45°, 90°, and 135° resulting in forty co-occurrence matrices. Second-order statistics, including
contrast, correlation, energy, and homogeneity were extracted from each co-occurrence matrix, producing a vector of four scalars for each pair of \((d, \theta)\). These vectors were subsequently averaged over all 40 \((d, \theta)\) values and considered as the feature representation for a parametric map.

For HistInt, even histogram bins in the range of [6, 20] were used. Consequently, one set of MMD values was derived on each HistInt computed at one of these bin sizes, and similar to the LBP method, the best bin size was selected by employing the SFS algorithm in a wrapper framework.

For different combinations of parametric maps, feature sets, and dissimilarity measures, the following naming convention was used in this paper:

[Parametric Map]–[Feature]–[Distance Measure]–[Kernel],

e.g., MBF–LBP–MMD–HIK.

The kernel field in naming is only associated with the MMD metric, and HIK was reported in the results due to its consistent superior performance on these data compared to radial basis function (RBF) kernel.

Simulations were performed on a Core i5-2520M machine with 8 GB of RAM, using 64-bit MATLAB (R2010b, MathWorks, USA).

3.C. Results of cell death categorization

To classify the cell death levels as low, medium, or high, the computed dissimilarity measures were submitted to a naïve Bayes classifier with thresholded cell death levels obtained from histopathology as the ground-truth labels. The cell death values were thresholded at two levels: 20% and 40%, and a binary classification was performed at each of these two levels to classify the cell death to less/more than the threshold (with low cell death <20%, medium 20%–40%, and high >40%). Classification was performed using leave-one-out scheme at subject level. Four measures were used to evaluate the classification performance of the CAP system including, accuracy, the AUC of the ROC, sensitivity, and specificity.

The classification results are graphically displayed in Fig. 5 for the two spectral parametric maps, MBF and SI, and two threshold values of 20% and 40% for different feature–dissimilarity combinations. For each feature set, the results of classification using MMD and \(\ell_2\) norm as two different dissimilarity measures are displayed pairwise for a better comparison.

There were immediate observations from the classification results. First, MBF proved to be more discriminating than SI, as can be judged from the performance measures. For example, the best performance achieved using MBF as the parametric map was an accuracy of 85.1% and 85.3% for 20% and 40% cell death thresholds, whereas the same on the SI parametric map was 79.9% and 80%, respectively. Second, in most cases, MMD outperformed \(\ell_2\) norm with an improvement of more than 10% in some cases (e.g., MBF–HistInt–LBP at 20% threshold). Third, LBP as a state-of-the-art texture method outperformed classical texture methods such as GLCM. Fourth, a combination of HistInt and LBP methods, especially on MBF parametric maps, led to better classification results than the individual feature sets. For example, the combination of the two features achieved an accuracy of 85.1% and AUC of 0.87 for the 20% cell death threshold on the MBF parametric map, whereas using the LBP alone as the feature extraction method achieved an accuracy of 77.0% and AUC of 0.77 for the same cell death threshold.

As a summary, the results in Fig. 5 indicated that the strongest classification performance was achieved using the MBF as the parametric map, combined LBP and HistInt as features, and the MMD as the dissimilarity measure.

3.D. Results of cell death prediction

The study on cell death classification to low, medium, or high using the proposed CAP system resembles more the clinical settings in which patients need to be categorized as responders or nonresponders, depending on cell death levels that can be judged by tumor shrinkage and cellularity.76,79 However, in preclinical studies, where the fraction of cell death is available from histopathological data, it is of great interest that the CAP system can also predict a continuous level of cell death. This objective was achieved in the proposed CAP system by replacing the classifier by a regressor and by using the continuous cell death levels as the ground truth instead of their thresholded values.

As depicted in Fig. 6, the plot of ground-truth cell death levels from histological analysis for all animals in this study is highly nonlinear. Therefore, a nonlinear regressor such as the SVR with a RBF kernel was employed for predicting the cell death levels. Similar to the classification study, the regression was performed in leave-one-subject-out (LOSO) scheme and the optimal trade-off parameter (\(C^*\)) and the optimal kernel width (\(\gamma^*\)) of the SVR were found using a grid search and fivefold cross validation on the training set in each fold of the LOSO. The performance of the CAP system in predicting the fraction of cell death levels was evaluated using the Pearson’s correlation coefficient (\(r\)),

\[
r = \frac{\sum_{i=1}^{n} (y_i - \mu_y)(\hat{y}_i - \mu_\hat{y})}{\sqrt{\sum_{i=1}^{n} (y_i - \mu_y)^2} \sqrt{\sum_{i=1}^{n} (\hat{y}_i - \mu_\hat{y})^2}},
\]

where \(n\) was the total number of animals, \(y\) and \(\hat{y}\) represented the actual (ground truth) and predicted fractions of cell death, respectively, \(\mu_y\) and \(\mu_\hat{y}\) were the means of those values.

Table I provides the results of cell death prediction using the proposed CAP system and the rival methods. The \(p\) values provided in Table I indicate whether the correlation between the predicted and ground-truth cell death levels was significant (\(p < 0.05\)). The results demonstrated that the LBP texture method outperformed a classical texture method such as GLCM, whose predicted cell death values never achieved a significant correlation with the ground-truth levels. Similar to the classification results, the MBF spectral parametric map performed better than the SI. Comparing MMD with \(\ell_2\) norm as the dissimilarity measure, MMD consistently outperformed (except for GLCM). Moreover, combining the LBP textural method with the HistInt was advantageous on the MBF parametric map.
The performance of the proposed CAP system in terms of classification accuracy and ±1 σ, AUC (the receiver operating characteristic), sensitivity, and specificity for the binary categorization of cell death levels to less/more than 20% or 40% using different feature-distance combinations on (a) MBF and (b) SI spectral parametric maps.

Fig. 5. The performance of the proposed CAP system in terms of classification accuracy and ±1 σ, AUC (the receiver operating characteristic), sensitivity, and specificity for the binary categorization of cell death levels to less/more than 20% or 40% using different feature–distance combinations on (a) MBF and (b) SI spectral parametric maps.
3.E. Statistical test of significance on treatment groups

Next, we compared the results of predicted cell death values with the ground-truth histological ones for individual treatment groups. As shown in Fig. 7 and described in full detail in Subsec. 2.B, the animals were divided to 27 treatment groups [3 treatment intervals (2/3, 24, or 72 h) × 3 microbubble doses (0%, 1%, or 3%) × 3 radiation doses (0, 2, or 8 Gy)].

Figure 7 depicts the histological and predicted cell death levels for the two best-performing approaches. The predicted cell death levels in Fig. 7(b) are those produced by the best feature–distance combination, combined HistInt and LBP feature methods with the MMD as the distance metric on the MBF parametric maps. Figure 7(c) shows the results for the second best performed approach, MBF–HistInt–MMD–HIK. Welch’s unpaired two-sample t-test$^{80}$ using a significance threshold of $\alpha = 0.05$ was performed between each treatment group and its corresponding control group (at the same post-treatment time interval).

It was observed from the histological cell death levels that 2–3 h after treatment was not sufficient to generate any significant changes in cell death levels. Also, neither ultrasound-mediated microbubble treatment administered alone (0 Gy) nor radiation therapy at single dose alone (no MB) had significant effects on cell death for any of the treatment time periods. However, a permutation combination of the two therapies resulted in significant changes ($p < 0.05$) in cell death levels, especially 72 h after treatment onset. An interesting aspect of the results was that a low dose radiotherapy at 2 Gy combined with US-stimulated MB at low concentration (1%) resulted in significant changes in cell death, whereas a high dose radiotherapy (8 Gy) alone did not.

The predicted cell death levels using the best-performed developed approach, consisting of combined features (LBP and HistInt), and the MMD metric on the MBF parametric maps closely followed the histological cell death values. In particular, it found resonance in significant cell death changes for the same treatment groups as histological cell death, with the exception of high-dose radiotherapy combined with high concentration MB after 2–3 h of treatment. This demonstrated that the developed CAP system based on spectral QUS methods in conjunction with advanced textural and machine learning techniques could predict cell death values with high accuracy at each treatment group. In contrast, the second best approach, based on MBF–HistInt–MMD–HIK [Fig. 7(c)], did not show significant changes in cell death for 2 Gy dose of radiation combined with low and high concentration MB 72 h after treatment. Therefore, the ultimate judgment on the acceptability of the $r$ values was based on whether an approach can correctly follow the ground-truth cell death levels for most of treatment groups, especially resulting in the same significance in cell death changes ($p < 0.05$) compared to the control in each treatment group. From this point of view, the strongest performance achieved by MBF–HistInt–LBP–MMD–HIK approach, as shown in Fig. 7(b), demonstrated an acceptable performance and the $r$ value achieved (0.6811) can be considered as a good predictor of cell death.

### Table I. The performance of the developed CAP system to predict cell death levels in terms of Pearson correlation coefficient ($r$). Histological cell death values were used as the ground truth. An SVR with an RBF kernel was used in a leave-one-subject-out scheme to predict the cell death levels. The highlighted entries indicate the maximum $r$ value achieved for a specific parametric map. The $p$ values indicate whether the correlation between the predicted and ground-truth values are significant ($p < 0.05$). * denotes $p < 0.05$; ** denotes $p < 0.01$; *** denotes $p < 0.001$.

<table>
<thead>
<tr>
<th>Param. map</th>
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<th>$p$</th>
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<td>0.005**</td>
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4. DISCUSSION

In this study, QUS spectroscopy methods in conjunction with state-of-the-art texture analysis and advanced machine learning techniques were used to develop a CAP system in order to categorize (discrete) and predict (continuous) levels of cell death, noninvasively, in wild type animals carrying fibrosarcoma murine tumors treated with ultrasound-mediated microbubbles as radiosensitizers. For the first time, this study...
demonstrated the viability of the proposed CAP system to classify the cell death levels as low, medium, or high, and also predict the continuous levels of cell death with high accuracy.

The earliest investigations on tumor classification using QUS biomarkers were able to successfully display the differences that arise in spectral parameters during cell death by using the mean of the parameters to represent an entire ROI.\[9,17\] Whereas the use of the mean of parametric maps does provide a means to interpret the status of the tumor cell over time, textural properties of parametric maps have shown a higher correlation with changes in the tumor tissue.\[57\] Specifically, texture properties derived from classical texture methods, such as GLCMs, demonstrated higher performance than the mean of intensity in response classification studies.
especially early on during a course of treatment. The reason for the improved classification performance when using more complex texture methods versus the simple mean method may be due to the additional consideration of structures (manifested to pixel intensities) next to each other within a sufficiently sized neighborhood. The LBP uniform patterns used in this study were primitive texture elements, such as spots, corners, edges, and smooth areas. As cell death happens due to treatment, the area inside the tumor becomes more heterogenous, and the existence of primitive textural elements such as spots, corners, and edges are speculated to become more prevalent, as opposed to the tumor before cell death, which is expected to mainly consist of smooth areas. Therefore, the LBPs are expected to be able to provide better discrimination as tumor morphological changes occur during the course of treatment administration, as demonstrated in this study.

The results indicated that state-of-the-art texture methods, such as local binary patterns, which provide a unified statistical and structural texture descriptor, outperformed classical texture methods such as GLCM. However, the LBPs are, by design, invariant to shifts in gray scale intensity levels, and therefore, they ignore the variations in intensity levels of 2D parametric maps between the pretreatment and post-treatment scans. These variations, however, convey useful information in cancer response monitoring, as demonstrated in the earlier works of QUS methods. The main reason is that different modalities of cell death increase the ultrasound backscatter, resulting in variations of MBF and SI parametric map intensity values. Therefore, including the gray scale information of parametric maps into the LBP method is expected to improve the discrimination power of the method in cancer response monitoring. This was implemented in this study by combining the features obtained from the histogram of intensity with the LBP features. The classification and predictions results, particularly on the MBF parametric maps, demonstrated the improvement of performance as a result of this combination.

The results of this study also demonstrated the advantage of using a kernel-based dissimilarity measure to quantify the distance between the pretreatment and post-treatment scans in the developed CAP system. A comparison between the results using MMD as a kernel-based metric with those using $\ell_2$ norm revealed that MMD outperformed the $\ell_2$ norm in most cases, sometimes by more than 10% of accuracy for the classification and more than 0.15 of the Pearson correlation coefficient ($r$) in prediction of cell death levels. For example, whereas the classification of cell death levels using MBF–LBP–MMD–HIK at 40% threshold resulted in 84.5% accuracy, 84.0% sensitivity, and 85.0% specificity, MBF–LBP–$\ell_2$ led to 73.8%, 76.0%, and 71.5% for the same performance measures, respectively [see Fig. 5(a)]. Similarly, according to Table I, using MBF–LBP–MMD–HIK for predicting cell death levels led to an $r$ value of 0.453, whereas using MBF–LBP–$\ell_2$ resulted in 0.281 for the same performance measure.

Overall, the strongest performance was achieved using the MBF as the parametric map, combined HistInt and LBPs as features, and the MMD as the dissimilarity measure. This combination achieved the highest performance in the classification of cell death levels at 20% (85% accuracy and AUC of 0.873) and very close to the highest performance at a 40% threshold (85.2% accuracy and AUC of 0.869). The combination also achieved the highest correlation coefficient ($r = 0.681$) in cell death prediction (Table I), and closely followed the histological cell death levels for all treatment groups indicating the same significant changes in cell death levels (Fig. 7).

In this study, fibrosarcoma murine tumor xenograft models in wild type animals were investigated in 27 treatment groups consisting of ultrasound mediated microbubbles alone, single doses of radiotherapy alone, and a permutation combination of the two aforementioned treatments in three different post-treatment timing intervals (i.e., 2–3, 24, or 72 h). It is worthwhile to mention here that since the earliest post-treatment imaging is 2–3 h after the injection of microbubbles, there is sufficient time for all microbubbles to be physiologically removed from the mice’s circulatory system (see Refs. 81 and 82 for a typical half-life of microbubbles, which is less than 15 min even without applying ultrasound stimulation), and therefore, they have no effects on the ultrasound imaging. The results of ISEL-based cell death quantization [Fig. 7(a)] demonstrated that ultrasound-stimulated microbubbles administered alone had minimal treatment effects resulting in no significant cell death compared to the control group at all post-treatment timing intervals. Slight increases in cell death, however, were noted for animals treated with higher microbubble concentration (3%), particularly 24 and 72 h after treatment. Also, single dose radiotherapy [no MB groups in Fig. 7(a)] did not result in significant increase in cell death compared to the control groups at any of the post-treatment timings, even at a higher dose of 8 Gy. However, ultrasound-mediated microbubbles combined with single-dose radiotherapy resulted in significant cell death, particularly when administered with higher-dose radiation (8 Gy) 24 and 72 h after treatment. Even a low concentration of microbubbles (1%) led to a significant increase in cell death levels when combined with 8 Gy radiation 24 and 72 h after treatment, and enhanced the effects of low dose radiation therapy at 2 Gy. The results also indicated that minimal effects could be obtained 2–3 h after treatment, suggesting that vascular effects due to injecting microbubbles stimulated by ultrasound may take up to 24 h to manifest into cell death. The findings presented here are in general agreement with previously published results.

The results of cell death prediction using the developed CAP system [Fig. 7(b)] were in close agreement with histologically detected cell death levels [Fig. 7(a)]. This suggests that QUS spectral methods, in conjunction with the proposed texture and machine learning techniques in this study, can be used in the future to assess the efficacy of cancer treatments. Considering that the area of computer-aided-prognosis for the assessment of cancer therapy effects is still in its infancy compared to computer-aided-diagnosis (CAD) systems, its counterpart for the purpose of cancer diagnosis, this work is a step toward the use of QUS technology in clinical cancer response monitoring.
In this study, an unpaired two-sample t-test was performed with a relatively small sample size in each treatment group. In order to evaluate the reliability of the performed statistical tests of significance, the statistical power (SP) was calculated. The results obtained indicated that using four mice per treatment group was adequate to achieve an SP above 80% for the ground-truth histological cell death levels. However, the results also indicated that it would be beneficial to increase the number of mice from 4 to 5 per treatment group in order to increase the SP threshold from 70% to 80% for the predicted cell death levels. This change would allow more concrete conclusions to be drawn from this study.

The proposed CAP system has the potential to be adapted to clinical applications for noninvasive therapeutic cancer response assessment, a step toward personalized medicine. To this end, in addition to the requirement of using QUS methods at conventional frequencies, some changes will be required to extend the proposed methods to patient data. These changes will include: performing image acquisition several times during the course of treatment, e.g., on weeks 1, 4, and 8, for longitudinal monitoring of the therapy effects; a different regime for histological analysis, as it only provides final treatment response, not the level of cell death during the course of treatment; and likely the requirement to employ techniques for learning from imbalanced data, as the number of responders is usually much higher than the number of nonresponders in clinical studies.

Several in vitro and in vivo studies have demonstrated that a nucleus is the major cause for ultrasound backscatter in high frequencies. At conventional frequencies, however, there is no such expectation to detect changes in backscatter intensity due to individual micron-sized particles. Instead, an aggregate change in the ensemble of cells and nuclei is hypothesized to influence acoustic properties and, consequently, ultrasound backscatter intensity, which are reflected in changes in MBF and SI in the same manner as those observed at high frequencies. Spectral slope, however, is related to the scatterer size. The nonsignificant change of slope has been reported in several QUS studies at conventional frequencies, which might be related to the existence of both small and large scattering structures that potentially play a role at these frequencies. For example, in xenograft tumor models with MDA human breast cell lines, in addition to small scattering structures (due to an ensemble of a few cells undergoing apoptosis), larger scattering structures, such as patches of response and developed gland-like features, could also affect the spectral slope in an opposite manner. These two different phenomena may cancel out their effects on the spectral slope and result in a different behavior compared to what is observed at high frequencies.

For the estimation of spectral parameters, the optimal RF block size is at least ten times the wavelength. The scatterers are different depending on the bandwidth and therefore, changing the bandwidth affects both QUS parameters and possibly texture parameters. However, the expectation is that the trend in changes due to cell death remains almost the same. Here, and in all other studies based on QUS methods, the focus has been on the changes in QUS texture patterns before and after the treatment, rather than on the absolute values. On the other hand, performing texture analysis at multiresolutions in this study enabled collecting the textural information at multiple scales. This has additionally made the designed CAP system more independent from the RF block size as changes that may occur in textural pattern sizes were well incorporated in the multiresolution texture descriptors. The sensitivity of the method to the RF block size and wavelengths is an interesting avenue for future research.

In this study, spectral QUS methods based on linear models fitted to the normalized power spectrum were used. In future, we will investigate the merit of more advanced QUS models, such as backscatter coefficient (BSC) parameters, computed by fitting a form factor (such as Gaussian model) to the normalized power spectrum in the application presented in this study.

5. CONCLUSION

In this study, a complete computer-aided-prognosis system was developed using QUS spectroscopy methods, a state-of-the-art texture descriptor based on predefined binary operators, and a kernel-based metric. The proposed CAP system was successfully employed in a longitudinal study of the enhancement effect of ultrasound-mediated microbubbles as radiosensitizers on radiation therapy in wild type mice carrying fibrosarcoma cancerous cell lines to noninvasively monitor and detect cell death fractions. The CAP system demonstrated the ability to categorize and predict cell death levels with high accuracy.

The developed CAP system provides an innovative approach to assessing treatment effectiveness early after the start of treatment in order to enable tailoring therapies to specific characteristics of individual — such as person’s genetic makeup, or genetic profile of an individual’s tumor — toward the goals of personalized (precision) medicine.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST DISCLOSURE

The authors have no COI to report.

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H. C. Kim, A. Al-Mahrouki, A. Gorjizadeh, R. Karshafian, and G. J. Czarnota, “Effects of biophysical parameters in enhancing radiation re-
A kernel based metric is a distance measure that has two properties: (1) it is kernel based, and (2) it is a metric. Kernel-based distance is a distance measure that has two properties: (1) it is a distance function $d(x, y) \geq 0$ (non-negativity), (b) $d(x, y) = 0 \iff x = y$ (identity of indiscernibles), (c) $d(x, y) = d(y, x)$ (symmetry), and (d) $d(x, z) \leq d(x, y) + d(y, z)$ (triangle inequality).


Out of 333 µs, only 32 µs was the tone burst and the rest was the gap and therefore, it resulted in a 10% ultrasound exposure.

Both B-mode and radiofrequency (RF) data were collected.


An alternative approach has been proposed in Ref. 55 with efficient linear time approximation, i.e., with a computational cost of $O(n + m)$ time. This is particularly important when larger volume of data is available. However, this is not the case in this study as there are limited number of scan planes per subject.


It is known from previous studies on QUS that ultrasound backscatter increases with apoptosis and this affects the level of MBF and SI parametric maps.


