On the Relationship between Dynamic Contrast-Enhanced Ultrasound Parameters and the Underlying Vascular Architecture Extracted from Acoustic Angiography

On the relationship between dynamic contrast - enhanced ultrasound parameters and the underlying vascular architecture extracted from acoustic angiography

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Abstract

Dynamic contrast-enhanced ultrasound (DCE-US) has been proposed as a powerful tool for cancer diagnosis by estimation of perfusion and dispersion parameters reflecting angiogenic vascular changes. This work aims at identifying which vascular features are mainly reflected by the estimated perfusion and dispersion parameters through comparison with Acoustic Angiography (AA). AA is a high resolution technique that allows quantification of vascular morphology. 3D AA and 2D DCE-US bolus acquisitions monitored growth of fibrosarcoma tumors in 9 rats. AA-derived vascular properties were analyzed

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along with DCE-US perfusion and dispersion in order to investigate the differences between tumor and control, and their evolution in time. AA-derived microvascular density and DCE-US perfusion showed good agreement, confirmed by their spatial distributions. No vascular feature was correlated with dispersion. Yet, dispersion provided better cancer classification than perfusion. We therefore hypothesize that dispersion characterizes vessels that are smaller than those visible with AA.
Keywords: Acoustic angiography, Dynamic contrast-enhanced ultrasound, Cancer, Dispersion, Perfusion, Ultrasound contrast agents

Introduction

Malignant tissue shows a set of alterations from benign tissue that can be used as markers to detect it (Koumoutsakos et al., 2013). Of particular interest for cancer imaging are the altered vascular architecture and the consequent changes in blood supply. Angiogenic vessels grow to nourish the tumor and support its proliferation. These vessels have been found to be tortuous, to grow chaotically, without the typical vessel hierarchy, and with a high occurrence of arteriovenus shunts. Many of these properties can be recognized with contrast-enhanced ultrasound techniques, which have shown promising results for distinguishing malignant tissue from benign (Brock et al., 2013; Gessner et al., 2013; Kuenen et al., 2013b, 2011; Mischi et al., 2012; Quaia, 2011; Shelton et al., 2015).

Dynamic contrast-enhanced ultrasound (DCE-US) captures the contrast-agent passage through the vascular bed after its injection in the patient's bloodstream. Specifically, it registers the local evolution of gray-level intensity at each pixel, referred to as the time intensity curve (TIC), which reflects the varying ultrasound contrast agent (UCA) concentration. The recorded intensities are then converted into UCA concentration with a linearization function specific to the employed ultrasound scanner (Rognin et al., 2008), yielding an indicator dilution curve (IDC) for every pixel in the video. Various characteristics of IDCs have been proven to be useful for distinguishing malignant from benign tissue (Mischi et al., 2012).

Several approaches have been adopted to extract information from IDCs derived from DCE-US bolus acquisitions. Some heuristic features of the IDCs, such as the wash-in time and the peak intensity, are related to cancer

(Mischi et al., 2012; Zhao et al., 2010). Multiple other techniques employ IDC fitting by analytical models, such as the lognormal, gamma, and local density random walk (LDRW) model (Strouthos et al., 2010). Functional parameters of the curves (e.g. area-under-the-curve) are extracted and displayed in colormaps, aiming to obtain a clearly distinguishable malignant region. All these approaches mainly attempt to quantify perfusion, which is motivated by the presence of ample arteries feeding the tumor, increased microvacular density (MVD), and presence of arteriovenous shunts. Despite this, clinical evidence has shown that cancerous lesions in the prostate can also be iso- or hypo-perfused (Brock et al., 2013). Indeed, it is known that tumor tissue has higher resistance to blood flow (Narang and Varia, 2011). This induces a couterbalancing factor that complicates predictions about the level of blood supply within the tumor, as compared to surrounding tissue (Cosgrove and Lassau, 2010). Furthermore, the MVD inside the tumor can be strongly heterogeneous, creating highly perfused regions as well as hypoxic, avascular regions. Therefore, assessment of perfusion alone is insufficient for reliable cancer diagnostics. These findings have motivated the development of contrast ultrasound dispersion imaging (CUDI), a method which enables assessment of UCA dispersion, in addition to quantification of perfusion (Kuenen et al., 2011; Mischi et al., 2012).

CUDI aims at quantifying the UCA dispersion due to the architecture of the vascular tree and complex multipath trajectories available for UCA transport. The main hypothesis that lies in the foundation of the method states that dispersion reflects structural vascular changes induced by angiogenesis. The first CUDI approach involved modelling of the IDCs in time domain with a LDRW model and extraction of a dispersion-related parameter from the fitted model (Kuenen et al., 2011). An important complication associated with this approach was poor signal to noise ratio, hindering the fitting procedure and decreasing its reliability. This problem has been mitigated by spatiotemporal similarity analysis (Kuenen et al., 2013a; Mischi et al., 2012). In a promising implementation, this approach involves calculation of an average correlation coefficient measuring the similarity of a TIC at a pixel and its surrounding pixels (Kuenen et al., 2013b). A theoretical description of the problem within the framework of the LDRW model has shown that the correlation coefficient between IDCs is monotonically related to the dispersion coefficient (Kuenen et al., 2013a). Moreover, this approach has demonstrated its superior performance compared to perfusion-related parameters at localizing prostate cancer in a clinical setting (Kuenen et al., 2013b). This method has been validated against cell differentiation reflected with the Gleason score for prostate cancer (Schalk, 2017). Another study identified that regions of low dispersion correlated with those of high MVD, quantified by immunohistology (Saidov et al., 2016). However, in this study detailed chatacterization of the vascular architecture (e.g. tortuosity and vessel size) was not available.

Acoustic angiography (AA) can provide accurate characterization of the vascular architecture: it is a high-resolution technique, capable of imaging individual microvessels (Gessner et al., 2013; Shelton et al., 2015). AA permits imaging vessels at a high resolution of 100-200 µm at 2 cm depth with minimal signal from tissue. While transmitting ultrasonic waves at frequencies in the order of a few MHz, close to the UCA bubble resonance frequency, it records the nonlinear response of the contrast agents in a high frequency range centered at 30 MHz. This technique grants the possibility to quantify vessel density and morphology measures such as the sum of angles metric (SOAM) and distance metric (DM) (Rao et al., 2016; Shelton et al., 2015). These parameters have been reported to be significantly different for malignant and benign tissue (Gessner et al., 2013; Shelton et al., 2015). Thereby, AA gives the opportunity to validate whether these features are reflected in DCE-US due to the different character of UCA perfusion and dispersion in these vessels.

The aim of this work is to determine whether DCE-US is able to characterize the underlying vascular architecture. It involves DCE-US and AA imaging of fibrosarcoma tumors and control regions in a longitudinal study of 9 rats. AA and DCE-US acquisitions were performed every 3 days, at 4 time points, starting with the day when the tumors could be palpated. An overall comparison of the tumor's and control's vascular properties was performed. Additionally, a longitudinal study of these properties was conducted, aiming to find similar trends in features extracted from the two different techniques of DCE-US and AA.

Materials and Methods

Rat Models

Fibrosarcoma tumor implantation was performed in rats according to a previously applied protocol (Streeter et al., 2011). The tumor models were established from propagated tumor tissue provided by the Dewhirst Lab at Duke University. Before surgery the (Fischer 344) rats were anesthetized with isoflurane; their left flank was then shaved and disinfected. An incision ($\sim$ 2 mm) was made above the quadriceps muscle, and a sample of tumor tissue ($\sim 1\ mm^3$) was positioned under the skin. The incision was closed with 1-2 staples. This procedure was performed at 3 different time points with 9 rats in total. Rats belonging to the same series were operated on the same day.

On day 8 after implantation, the first ultrasound acquisition was performed if the tumors were palpable. Otherwise, we waited for 2-3 days for subsequent assessment. When the tumors were palpable, UCA was injected in the rats' tail vein through a 24 gauge catheter while the animals were anesthetized with vaporized isoflurane in oxygen. DCE-US was performed on the tumor-bearing flank for assessment of perfusion and dispersion. The AA acquisition protocol immediately followed the DCE-US acquisition to minimize the amount of time each animal spent under anesthesia. The beginning of the DCE-US and AA acquisitions were different between the series, starting with day 8, day 11, and day 13, respectively. For all but one animal, subsequent imaging acquisitions were performed with an interval of 3 days, amounting to 4 time points in total. One rat was an exception since we were not able to inject the contrast (for both modalities) in its tail vein, and managed to image only at the first and third time points. All experiments were performed at the University of North Carolina at Chapel Hill, approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill.

Image acquisition

DCE-US bolus injection protocol

A UCA bolus of 2×10^8 microbubbles was injected in the rats' tail vein. The contrast agent used in this study was made in-house; it has a lipid shell and perfluorcarbon core, similar to Definity® (Latheus Medical Imaging/U.S.A, N. Billerica). A 15L8-S probe was utilized with a Siemens Sequoia scanner in Cadence Pulse Sequencing mode at an insonifying central frequency of 7 MHz. The acquired DCE-US recordings were stored in DICOM format.

AA continuous infusion protocol

A continuous infusion of microbubbles was administered using a syringe pump (PHD 2000, Harvard Apparatus) at a rate of 1.5×10^8 microbubbles per minute. AA imaging was performed with a dual-frequency single-element transducer transmitting at 4 MHz, and receiving around 30 MHz. The 3D AA images were acquired plane by plane, with a step size of 100 µm.

DCE-US bolus data processing

Preprocessing

All the bolus recordings were filtered with a Gaussian filter, as previously performed in (Mischi et al., 2012), using a kernel of 0.13 mm equal to 1.6 pixels. This value improved the signal-to-noise-ratio at the cost of additional spatial correlation between TICs at neighbouring pixels. The TIC power of every pixel was evaluated as the root mean square of the TIC after the baseline was removed. Regions with a level of TIC power below -22 dBs of the maximum TIC power over all images were excluded from further analysis (shown in black in Fig. 1 a.). This limited the effect of random noise on the parameters of interest (Kuenen et al., 2014). Characteristic of DCE-US is multiplicative noise: noise proportional in its power to the signal amplitude. By eliminating regions with low TIC power, we avoided erroneous parameter estimation from regions with low signal power where random noise dominates. After this, the intensity values of the remaining regions were linearized by inverting the logarithmic compression function implemented in the adopted scanner, yielding the IDCs.

Assessment of dispersion

An average correlation coefficient was calculated for every pixel between its own IDC and those at its surrounding pixels within a ring-shaped kernel (Mischi et al., 2012) with an inner radius of 0.6 mm and an outer radius of 2 mm. The inner radius was chosen equal to the lateral resolution of the preprocessed bolus recordings at $\sim$ 2 cm depth as identified with local autocorrelation analysis. Details about the latter procedure can be found in (Mischi et al., 2012). The lateral resolution was taken as a reference since it was worse than the axial resolution. The outer radius of the kernel was set equal to the size of 2 mm, which a tumor can usually reach without neovascularization (Folkman, 1971). The time window over which the IDCs were correlated to each other was selected to maximize the area under the receiver operating characteristic curve for tumor classification, resulting in a value of 17 seconds as proposed in previous work (Panfilova et al., 2016). This is the only informative segment of the IDC (Fig. 2) due to early recirculation, as often observed in small animals (Stapleton et al., 2009). In this work, the beginning of the analyzed time window was set with 3 seconds before the appearance time, ensuring the wash-in phase to be entirely captured.

Assessment of perfusion

Wash-in-rate was adopted to assess perfusion and computed as the slope of a line fitted to the IDC in the 2-second interval after appearance time, as illustrated in Fig. 2. The value of 2 seconds was chosen to reflect the rise of UCA concentration in the initial part of the IDCs in all acquired clips.

AA data processing

The AA volumes were interpolated to reduce the inter-plane distance to 50 microns and make the pixels isotropic. Visible vessels were manually segmented and characterized in terms of vessel dimensions: vessel length (VL) and mean radius (MR). VL was computed as the length of the vessel segment identified between successive branching points, and MR was computed as the mean radius of this vessel segment along its length. Vessel tortuosity was assessed with the distance metric and the sum of angles metric (Bullitt et al., 2003). The DM was computed as the ratio of vessel length to the Eucledian distance between its beginning and end. The SOAM was calculated as the sum of angles between successive points on the vessel centerline divided by VL, using the same formula as in (Bullitt et al., 2003), but excluding the torsional angle. Besides these individual vessel properties, MVD was calculated as a global characteristic of the tumor at a given timepoint, defined as the number of visible vessel segments divided by tumor volume. The volume vascular density (VVD) was computed with a moving 3D isotropic kernel in the central slice of the tumor ($\sim$ 1 mm in thickness). Otsu's method (Vala and Baxi, 2013) was used to select a threshold to separate noise from vessel signal within the central slices; the percentage of pixels with vessel signal from the overall number of pixels in the 3D kernel was calculated.

Statistical analysis

The DCE-US parameters were spatially downsampled by a factor 7 in both directions, equal to the resolution of the preprocessed images. This was performed to exclude spatial correlation and prepare the data for the statistical tests that require sample independence.

Comparison between tumor and control

Dispersion and perfusion values were divided into two groups. The tumor group was composed of the manually selected tumor regions (inside the red contour, Fig. 1 a.) from all rats at all time points binned together. The control group was taken from pixels outside the tumor contour, dilated by $\sim$ 1 mm (in blue, Fig. 1 a.). The region between the red and blue contour was excluded from analysis to avoid erroneous pixel assignment to tumor or control, since DCE-US information was not considered sufficiently comprehensive for such accurate tumor delineation, as required by e.g. ablation therapy and surgery. The AA parameters were extracted in a similar fashion: vessels were taken from within the tumor region and outside it in the same flank (Fig. 3). Vessel segments on the border of the selected contour, whose belonging to a tumor or control group was debatable were disregarded from analysis.

An Anderson-Darling goodness of fit hypothesis test was performed on all the parameter distributions to check for data normality. Since all the distributions were identified as non-Gaussian, a Mann Whitney non-parametric test was performed to establish the significance (p-value) of the difference between tumor and control. No additional subsampling or upsampling was performed to make the control and tumor data sets balanced, since the Mann Whitney test can be applied to data sets with distributions of different size (Mann and Whitney, 1947).

The Cohen's d was used as a measure of the 'effect size' (Sullivan and Feinn, 2012) that the tumor has on the underlying vasculature, calculated as the difference between the means of two distributions divided by the standard deviation of the control. The values of the Cohen's d term allow to classify the difference between two distributions according to 4 categories: small, medium, large, and very large for values of 0.2, 0.5, 0.8, 1.3, respectively.

Longitudinal study of tumor and control

A longitudinal study of the tumor evolution was performed with the Kruskall-Wallis post hoc test, evaluating the differences among the distributions of dispersion and perfusion, and vascular features of tumor and control at 4 time points. The Kruskall-Wallis test (Kruskall and Wallis, 1952) does not require equal sample sizes, which is an advantage considering that our data set is unbalanced and incomplete: data is missing for one tumor at two time points as well as control at several time points for the large tumors. Moreover, the number of visible vessels is different for every image acquisition. For all rats, all parameter values were binned together according to the time point of the acquisition.

The statistic test calculation is influenced by the number of observations and can result in different outcomes for different sample sizes (Kruskall and Wallis, 1952). Since the number of pixels provided more samples for dispersion and perfusion compared to the number of vessels extracted with AA, these pixels were randomly subsampled to yield the same number of samples as vessels per each representative dataset of tumor and control at each time point. The only parameter that remained different in terms of group size is the MVD; being a global parameter that characterizes the entire tumor and control at a specific time point.

After the post-hoc tests were performed, the Pearson correlation coefficient was computed between the medians of the parameters showing similar longitudinal trends.

Mapping of vascular properties on the bolus acquisition plane

During the DCE-US bolus acquisistions the operator always tried to image the largest cross-section of the tumor, and keep the same orientation of the probe as used for AA. However, it was noticed that these precautions were not sufficient to reliably identify the DCE-US plane within AA: even a movement of the order of ~ 1 mm alters the imaged vascular pattern of a tumor. It was noticed that the perfusion maps highlight larger vessels, clearly visible in the AA (Fig. 2 b. and d.). These vessels were used as markers to locate the bolus recording plane in the AA volume. For this, a dedicated tool was developed, allowing to freely scroll through the AA volume planes and change their orientation.

The selection of the plane was performed by visual inspection, choosing an image containing as many as possible vessel markers present in the perfusion maps. A slice in the AA volume of ~ 1 mm thickness was selected and an extention of the skeletonization algorithm described in (Meiburger et al., 2016) was applied to extract MVD (Fig. 2 e.), MR (Fig. 2 f.), VL, and

SOAM. This slice thickness was chosen to be of the order of the elevational resolution in the bolus recordings and sufficiently large to register vessel segments. This allowed a qualitative comparison of the spatial distribution of the vascular features with those of dispersion and perfusion in the same plane.

All the image processing and statistical analysis was performed with Matlab software (the MathWorks, Natick, MA).

Results

Statistical analysis

Comparison between tumor and control

For all the extracted parameters, tumor and control have significantly different distributions ($p < 0.001$). However, the magnitude of the differences, expressed in Cohen's d, spans a wide range (Fig. 4), showing a marginal effect size for the DM (Fig. 4 c.), and small to very large differences for the rest of the parameters.

Longitudinal study of tumor and control

Since the DM showed almost no difference between tumor and control, it was excluded from the longitudinal analysis. Boxplots with all parameter values binned according to the time points are shown in Fig. 5, while Fig. 6 illustrates the results of the post hoc Kruskall-Wallis test, color-coded according to the significance level of the intra-distribution differences.

The dispersion median is relatively constant in time for both tumor and control, showing a significant difference for control and tumor distributions

(Fig. 5 a., Fig. 6 a.). Tumor perfusion is significantly different from the control at all time points (Fig. 5 b., Fig. 6 b.), peaking for the tumors at the second time point. Interestingly, the longitudinal trend of the control's perfusion seems to mimic the tumor's trend in time, however, at a smaller magnitude, not identified as significant with the post hoc test.

The VVD is stably higher for tumor, while the MVD seems to follow a similar trend to that of perfusion, peaking for tumors at the second time point. However, the result of the MVD post hoc test is difficult to compare to others since the number of samples is different: only one value of MVD per time point is available, while the other parameters were subsampled according to the number of segmented vessels in the AA volume at a given time point.

The post hoc results, illustrated by colormaps in Fig. 6, are comparable for dispersion, the VVD, the VL, and the SOAM. However, no significant correlation between the medians of the dispersion levels and the mentioned AA parameters has been identified. As for perfusion, the mean perfusion in tumors and their MVD showed a significant correlation coefficient of 0.572 ($p < 0.001$) and inclusion of both control and tumor values resulted in a Pearson correlation coefficient of 0.67 ($p < 0.001$).

Mapping of vascular properties on the bolus acquisition plane

The spatial parametric maps of the AA skeleton confirmed our observation that there is a correlation between regions of high perfusion and elevated MVD (Fig. 1 b. and f.). No spatial correspondance was found between dispersion and the other AA - derived parameters.

Discussion

Dispersion shows a large difference (Cohen's d = 1.68) between tumor and control, exhibiting stable performance at tumor detection as it develops. Perfusion shows a lower discrimination power than dispersion, that is high for younger tumors, peaking at time point 2, and decreases with tumor growth. Interestingly, the perfusion level in the control around the tumor is also elevated (Fig. 5 b.), showing a similar trend as in the tumor itself. This may reflect that the overall perfusion of tissue around the tumor is increased and influenced by the tumor. This effect has been shown for the SOAM, which exhibits intermediate values between that of tumor and control in tissue adjacent to the tumor (Rao et al., 2016). Moreover, it has been shown for the fibrosarcoma model that the vascular source is often located in the periphery of the tumor (Ponce et al., 2007; Tozer et al., 1990; Viglianti et al., 2004).

Dispersion of the control stays stable over time, indicating that dispersion-related changes mainly occur within the tumor itself, and not in the surroundings. The spatial perfusion and dispersion maps are complementary, showing different patterns of highlighted regions (Fig. 1 b. and c.). Perfusion highlights large vessels, as well as regions with high MVD.

The SOAM indicates that the tumor has more tortuous vessels, exhibiting a similar trend to that of dispersion (Fig. 5 a., g.) and comparable results for the post-hoc test (Fig. 6 a., g.). Nevertheless, the effect size difference, as indicated by Cohen's d, is much lower for the SOAM than for dispersion. In general, the control regions in this experiment show a higher tortuosity than we previously observed for these rats, expressed by the DM in (Shelton et al., 2015). Direct comparison of the SOAM in this work and in (Shelton et al., 2015) is not availible since the calculation of the SOAM has been adjusted since then. The unusually high tortuosity for control may be caused by the presence of the bowel region in some of the AA images, which was excluded from analysis in earlier studies, and may have elevated tortuosity. Previous data also shows that the SOAM exhibited an intermediate level of tortuosity in tissue up to 1 cm away from a tumor, with a mean tortuosity between that of tumors and non tumor-bearing animals (Rao et al., 2016). The discrimination power of the SOAM in our data set increases for smaller vessels (Cohen's d= 0.14 for vessels with a radius $>$ 0.11 mm, 0.28 with an intermediate radius, and 0.43 with a radius $<$ 0.09 mm). Therefore, its relation with the extracted DCE-US features can not be fully appreciated due to the finite resolution of AA. Similarly, a previous study has shown that the difference in MVD between tumor and control increases for smaller vessels (Sedelaar et al., 2001). Therefore, it may be that the SOAM, MVD, and other metrics extracted in this study are related to dispersion; however, mainly smaller vessels' properties have a significant influence on it. Supporting this hypothesis is the former observation that regions with increased MVD correspond to those with low dispersion (Saidov et al., 2016), as derived from immunohistology. The immunohistology derived MVD was based on evaluation of tomato lectin binding to the endothelial cells and therefore characterized the presence of vessels of all sizes.

Spots of increased vascular density or large vessels were detected with perfusion colormaps. The correlation between median perfusion level and MVD is the only significant inter-parameter agreement found in this work.

The Kruskall-Wallis test is ideally constructed for a study design when subjects are randomly assigned to different groups, so that each subject appears in one group only (Kruskall and Wallis, 1952). Moreover, the subjects within the group must be independent. We realize that these assumptions are not strictly valid in this study, since we observe the tumor evolution in the same rats over time and since the vessels selected from the same rat are, strictly speaking, not independent. However, we do not expect these limitations to be crucial for deriving a meaningful conclusion about the significant trends in time.

Imaging initialization was different among 3 series of experiments, starting with day 8, 11, and 13 after tumor implantation, as explained before. We consider that combining all the rats together according to the number of the acquisition is justified as the imaging was initialized according to the same strategy: when the tumors became palpable. However, since we waited for 2-3 days for subsequent assessment if tumors were not pulpable on day 8, in future work it may be benifitial to assess the tumors every day or evaluate all tumors in a single cohort. This would ensure that the development of the imaged tumors is more consistent.

It is often observed that the wash-out phase is masked by recirculation in small animals. (Stapleton et al., 2009) shows that for a range of administered UCA doses the wash-out phase is more prominent in mice. Different UCA doses should therefore be investigated in our future work, since a prominent wash-out phase, in our experience, enhances the performance of CUDI (Kuenen et al., 2013b). A clear wash-out would also allow evaluating the wash-out as a complementary perfusion parameter.

An important limitation of this study is the 2D character of the extracted parameters of dispersion and perfusion. The results of the post hoc tests, therefore, must still be taken with caution since it was performed for 3D vascular features evaluated in the whole tumor volume and 2D dispersion and perfusion that leave us blind to out of plane information and restrict us to the central tumor slice, which is not always representative of the whole tumor (Streeter et al., 2011). We mitigated this limitation by performing an additional spatial comparison of the parameter maps in the same plane, matched with the help of large vessels identified in the perfusion maps. The agreement between perfusion and MVD, noticed in the longitudinal trends, was also identified in the spatial distribution of these parameters in the same plane, raising more confidence to the finding that perfusion and MVD are correlated.

An improved study design should either include 3D DCE-US (Schalk et al., 2015), giving more accurate overall tumor characteristics, or a registration procedure, allowing to fix the orientation of the probes and identify the location of the DCE-US plane within the AA volume. The finding that perfusion highlights large vessels can be used to further improve registration.

The abscence of any parameters correlated with dispersion may pinpoint to the limitation of AA as a validation method for CUDI: while enabling very high resolution ultrasound imaging, it may not be sufficient to find out which vascular properties substantially influence dispersion, since dispersion may be mainly defined by properties of subresolution vessels. In this respect, it is possible to direct our attention to superlocalization methods that overcome the limit of diffraction: they are able to track singe bubbles and determine their exact positions by finding the centers of their point spread functions (Cox and Beard, 2015; Errico et al., 2015). Another possible reason for the abscence of vascular parameters that correlate with dispersion is that the adopted dispersion parameter, is in fact related to both dispersion and flow velocity (Kuenen et al., 2013a). Different vascular parameters may contribute to the separate terms of dispersion and flow velocity, while we assessed their combination. In this regard, it would also be of interest to apply another analysis to the DCE-US bolus recordings that allows to separate dispersion and velocity contributions (van Sloun et al., 2017).

Conclusions

In this work, dispersion demonstrated its superior performance at tumor classification compared to perfusion, as previously found for prostate cancer (Kuenen et al., 2013a,b; Mischi et al., 2012). Perfusion colormaps highlight large vessels and regions of elevated MVD. The vascular factors that determine the dispersion level remain yet to be found, as well as the role of vessels with a diameter below 100-200 µ in defining perfusion levels.

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and a co-founder of SonoVol, Inc, a company which has licensed this technology.

Abbreviations

AA Acoustic angiography

CUDI Contrast ultrasound dispersion imaging

DCE-US Dynamic contrast-enhanced ultrasound

DM Distance metric

IDC Indicator dilution curve

LDRW Local density random walk

MR Mean radius

MVD Microvascular density

SOAM Sum of angles metric

TIC Time intensity curve

UCA Ultrasound contrast agent

VL Vessel length

VVD Volume vascular density

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Figure Captions

Figure 1: DCE-US and AA images of the same plane, and maps of the extracted features. a: maximum intensity projection of the DCE-US video. The tumor is encircled by a red contour, while the region outside the blue contour belongs to the control, separated by a margin which was not included in the analysis. Regions with power below the threshold of -22 dBs of the maximum intensity are displayed in black. b-c: perfusion and dispersion colormaps, respectively. Regions with power below -22 dBs of the maximum intensity are displayed in white. d: Selected AA slice. e-f: vascular skeleton, colorcoded according to the values of the microvascular desity, and mean radius, respectively (yellow indicates low values, while red inicates high values). The numbers in b and d illustate the vessels identified in the perfusion maps, used as markers to locate the right plane in AA volumes.

Figure 2: A typical preprocessed indicator dilution curve. T1 shows the appearance time, T0 is taken 3 seconds before appearance time. The interval from T0 to T2 shows the interval of the IDC used for dispersion analysis. The tangens of the angle alpha of the line fitted to the indicator dilution curve in the 2 seconds after appearance time is the wash-in-rate.

Figure 3: AA maximum intensity projection. The tumor region is indicated by the red contour, surrounded by the control region.

Figure 4: Boxplots of tumor and control parameters, binned together from all time points. a: dispersion, b: perfusion, c: distance metric, d: sum

of angles metric, e: vessel length, f: vessel radius, g: microvascular density, h: volume vascular density. Cohen's d measure is indicated above the plots.

Figure 5: Boxplots of tumor (T1, T2, T3, T4) and control (C1, C2, C3, C4) parameters, binned together at different time points. a: dispersion, b: perfusion, c: volume vascular density, d: microvascular density, e: vessel radius, f: vessel length, g: sum of angles metric.

Figure 6: Results of the post hoc Kruskall-Wallis test performed on tumor and control parameters at four time points (indicated by T1, T2, T3, T4 and by C1, C2, C3, C4, respectively). The colors of the rows indicate whether the distribution is significantly different from the others, green and yellow representing different significance levels. a: dispersion, b: perfusion, c: volume vascular density, d: microvascular density, e: vessel radius, f: vessel length, g: sum of angles metric.