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Rapid 3D Imaging of Contrast Flow: Application in a Perfused Kidney Phantom

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Abstract

Previous studies indicate imaging of ultrasound contrast in 3D is potentially superior to 2D imaging for vascular characterization. A dual beam, dynamic refill technique, which relies on volumetric contrast clearance and sequential imaging, was used to image a preserved porcine kidney perfused with contrast. A model was developed for the contrast profile across the renal artery in order to estimate fractional blood volume. This model was used along with refill curve information to measure absolute perfusion within renal cortex for a 100-cm³ volume. Perfusion measurements from a slice within the volume were also made using a modified interval imaging technique. The measured perfusion using the dual beam technique was consistent with the perfusion measured using the interval imaging technique (dual beam values were 1.06 ± 0.04 times corresponding interval imaging values). These experiments suggest that ultrasound contrast perfusion measurements are independent of the volume of contrast eliminated prior to refill.

Keywords

contrast refill; perfusion; blood flow; imaging; vasculature; time-intensity curve

Introduction

Ultrasound imaging of vasculature in a three-dimensional volume may allow superior characterization of tissues when compared to two-dimensional imaging (Carson et al. 1997). In addition, visualization and mapping of the blood flow within and surrounding tumors may provide a means for differentiating between benign and malignant masses (Bhatti et al. 2001; LeCarpentier et al. 1999; Sehgal et al. 2000). High-frequency (circa 30 MHz) 3D Doppler imaging has been used to visualize tumor vasculature (Xuan et al. 2007); however, the high frequencies preclude the imaging of nonsuperficial tissues. At lower frequencies, contrast enhanced ultrasound (CEUS) provides a means for increasing the range of vessel sizes and flow rates that can be included in such evaluations relative to Doppler imaging. In addition, contrast transit dynamics can be obtained since after contrast is cleared from a

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volume, its refill can be monitored. For any given region of interest, signal intensities can be plotted as a function of refill time and fitted to a time-intensity curve from which the contrast mean transit time (MTT) can be estimated (Kamiyama et al. 1999; Moriyasu et al. 1996; Ohmori et al. 1997; Wei et al. 1998).

The dual-transducer (DT) technique illustrated earlier (Chen et al. 2007) is one dual-beam method for collection of three-dimensional data. Sweeping a transducer pair, composed of a clearance transducer and a low-power imaging transducer as a unit at velocity v over a contrast perfused tissue volume collects the refill images of the entire volume at a given refill time as shown in Fig. 1. Slice separation is determined by both the trigger rate of the imaging transducer and the translation velocity v . The DT technique provides a means for measuring the contrast replenishment curve following clearance of contrast from a large volume. Interval imaging (II), on the other hand, involves clearing a thin tissue volume of contrast prior to image acquisition. The development of 2D arrays would allow increases in the volume of contrast clearance zones relative to that used in II, and this increase could cause differences in estimated perfusion as an artifact. This study investigates the DT method in measuring perfusion comparison to that obtained from II.

In order to examine possible effects of the size of the contrast clearance zone on perfusion estimates, a preserved porcine kidney is used as a three-dimensional phantom (Holmes et al. 1984). Such a phantom contains a vascular bed and provides an environment to use the dual beam method with a more realistic spatial distribution of flow than the cylindrical tube phantom previously reported (Chen et al. 2007). Comparison of perfusion estimates obtained from dual beam acquisitions with those using II can be used to detect differences arising from the change in the contrast clearance zone volume.

Methods

Theoretical Considerations

In a perfused vascular bed, contrast agent fills the blood vessels, and it is assumed that the agent follows the fluid flow perfusing the bed. Immediately after contrast clearance and the cessation of the high-powered insonation, contrast from outside the disruption zone begins to refill the cleared volume. The refill can be measured either continuously with low-power pulses (real-time refill imaging) (Schlosser et al. 2001) or intermittently (Kamiyama et al. 1999; Moriyasu et al. 1996) at only specified times. The contrast signal intensity (assumed proportional to the contrast concentration) (Marsh et al. 1998) at the measured time points can then be fitted to an exponential equation of the form $y(t) = A[1 - \exp(-t/MTT)]$ after subtraction of the tissue baseline, where A is the signal intensity at the asymptotic limit, t the time interval, and MTT the mean transit time (Wei et al. 1998). Other models have been previously described such as the error function fit (Potdevin et al. 2004; Arditi et al. 2006), which systematically accounts for the elevational beam shape, and the sigmoidal function model (Lucidarme et al. 2003). We have chosen here the more commonly used exponential model in order to facilitate comparison with other studies.

A modified II technique (low-power II) allows the acquisition of interval imaging data at minimally destructive acoustic power for comparison with DT acquisitions. As illustrated in Fig. 2, the insertion of a single low-power frame immediately before the contrast clearance sequences provides a series of low-power images corresponding to the same flow being monitored by the traditional II sequence.

For the purposes of this paper, perfusion is defined as the volume of fluid per unit time passing through a unit volume of tissue. In the fitted exponential expression after baseline subtraction, the asymptotic value A divided by the MTT is indicative of perfusion (reviewed

in Fleischer et al. 2004). Dividing A by the signal intensity of 100% contrast agent suspension $A_{100\%}$ gives the moving fractional blood volume (FBV). The perfusion through any given tissue is FBV/MTT , which can be expressed in units of inverse time. Forsberg et al. (2006) have previously estimated perfusion from the initial slope of time-intensity curves normalized to $A_{100\%}$ following contrast bolus injections. Waller et al. (2007) have examined the upslope of time-intensity curves, in addition to other parameters, in distinguishing renal cortex from medulla in canines. Similarly, perfusion can also be estimated from the initial slope of the normalized exponential refill curve, which equals $r = FBV/MTT$, in the case of continuous contrast infusion.

Experimental procedures

Ultrasound scans were conducted within a tank lined with 13 mm thick sound absorbent ultra-soft polyurethane (Sorbothane Inc., Kent, OH, USA) and filled with water that had been allowed to come to gas saturation. A schematic of the experimental setup is shown in Fig. 3. Each motor turns a threaded rod to translate a platform with an attached transducer holder. Motors are connected to a control module (Microkinetics Corporation, Kennesaw GA, USA) containing the electronics driving the motors. The control module contains circuitry needed to operate the footswitch trigger and is linked to the footswitch control of the GE Logiq 9 (General Electric Corporation, Milwaukee, WI, USA) in order to trigger image acquisition from the imaging transducer. The DAQCard-700 (National Instruments Corporation, Austin, TX, USA) linked the module to a laptop computer that controlled transducer motion and triggering through a LabVIEW (National Instruments) script. The kidney phantom was mounted beneath the water on a stand and secured with two elastic bands. As previously described (Chen et al. 2007), a DT assembly was used to demonstrate the dual beam technique by controlling both transducer separation and translation along the transducers' elevational direction. Although two transducers were used for acquiring the volumetric images in this work, any setup composed of two independent beams, such as a two-dimensional array, could be used. The transducer assembly was mounted above the kidney being scanned.

A fresh porcine kidney was obtained from a local supplier and preserved as described in the literature (Holmes et al. 1984). Subsequently the kidney was rehydrated and degassed by perfusion through the renal artery with (> 5 L) degassed water. The kidney was then perfused with a 1:5000 suspension of Definity (Bristol-Myers Squibb Medical Imaging, N. Billerica, MA, USA) at 17 mL/min and 33 mL/min (referred to here as the slow and fast rates, respectively) using a peristaltic pump (Cole-Parmer L/S pump and drive, Cole-Parmer Instrument Corporation, Barrington, IL, USA). DT scans in the transverse orientation as shown in Fig. 4 were made with the Toshiba Powervision (Toshiba America Medical Systems, Tustin, CA, USA) system using the PVN-375AT transducer for contrast clearance. A constant power output and firing rate were maintained by placing the Toshiba system transducer in harmonic mode (power setting of P16 (MI=1.0) at a frame rate of 127 Hz and a center frequency of 2.3 MHz). The GE Logiq 9 (General Electric Corporation, Milwaukee, WI, USA) in phase inversion (PI) mode was used at low power (1% acoustic output-AO MI=0.05) with the 7L transducer to image at a receive center frequency of 7.0 MHz.

Images for a 42 mm thick volume were acquired for refill times of 8, 10, 15, 20, 25, 30, 40, 60, 70, 120, and 150 s with a 1 mm slice separation using the DT apparatus ($n=4$ for each flow rate, with the exception of the 150 s point for 2 acquisitions at the fast flow rate due to technical difficulties). The scans encompassed a total phantom volume of 100-cm^3 , with each image slice being approximately 4.7 cm in the lateral direction and 5 cm in the axial direction. The GE Logiq 9 was specifically adapted to store triggered images into the cine buffer through its footswitch control. A LabVIEW script (National Instruments Corporation, Austin, TX, USA) was used to both control transducer translation and slice separation via

the DAQCard-700. The transducers were translated at a speed of 6 mm/s, which was the maximum translation speed attainable with this apparatus. The 1 mm slice separation was obtained by triggering a solid-state relay (PVN 012-ND International Rectifier, El Segundo, CA, USA) linked to the GE Logiq 9 footswitch control at 6 Hz. The acoustic output of the clearance transducer was manually stopped at the start of image acquisition in order to eliminate transducer cross-talk.

Refill data using II (n=5 for both flow rates) were collected using the GE L9 at a transverse slice position (20 mm from the start of the acquired volume) with the same orientation for comparison to DT results. The 20 mm position was near the center of the cleared volume; therefore, any differences in measured perfusion arising from differences in contrast clearance zone thickness would be more pronounced than in cases where the position was further from the center. The refill times acquired using II were also the same as those acquired with the DT technique, except for the substitution of 5 s in place of 8 s and the lack of a 10 s point for one fast flow acquisition due to technical difficulties. Using the footswitch trigger, twenty-five clearance pulses at 5 Hz were fired at high power (100% AO MI=0.4) and low-power II frames were acquired by observing contrast signal for different intervals at 1% AO immediately prior to contrast clearance. The low-power II frames were used exclusively because they were obtained at the same AO as the DT measurements. All acquired images were decompressed to a linear echo power scale prior to analysis. Due to variations in the contrast agent during the course of the acquisitions (details in appendix A), II subtraction images were computed at each time point by subtracting the low power baseline tissue image acquired at the end of the contrast clearance sequence from the corresponding refill image, and recompressing to a log power scale. DT subtraction images were computed similarly by subtracting the mean baseline tissue image (average of all the II baseline images) from the raw DT image, and again recompressing.

Contrast variation and normalization

Variations in concentration of the infused contrast agent suspension alter the signal intensity observed during the course of refill. These variations include both differences in initial concentration, as well as any decay over the course of acquisition. Agent variation within a refill curve acquisition distorts the curve, and if ignored would yield erroneous flow parameters. Properly normalizing the signals obtained from the cortex to the input contrast agent should correct for contrast agent variation. In addition, such normalization would allow estimation of the FBV and perfusion (perfusion being $r = \text{FBV}/\text{MTT}$). The input contrast agent can be monitored through any relatively large blood vessel with a high flow rate. Due to its large size and high flow velocity, one can assume that the renal artery is always filled with contrast whenever the kidney is being perfused regardless of the refill state of the remainder of the kidney. Therefore, the renal artery was used as a reference vessel to normalize measured cortex signals to account for contrast variability.

The normalization process must account for the following factors: (1) overlying attenuation, (2) reference vessel size, and (3) reference vessel position. First, any difference in overlying attenuation between the renal artery and cortex region of interest (ROI) would distort the signal intensity of one relative to the other. Both the renal artery and cortex ROI, denoted in Fig. 5, however, have negligible overlying attenuation; therefore overlying attenuation does not play a significant role for this specific study. Second, the signal intensity within the reference vessel can be applied to a model described below to estimate the intensity A of 100% contrast suspension at the vessel depth. The model accounts for effects arising from vessel size. Third, since the reference vessel is located at a different depth than that of the kidney cortex, A needs to be scaled in order to account for signal intensity changes from differences in beam geometry and imaging system parameters.

Reference artery contrast model

As indicated above, a reference vessel signal allows for normalization but must be treated properly to account for specific factors affecting the reference signal level to be used. For the purposes of this work, the reference artery was modeled as an in-plane cylindrical tube with a fixed radius a and angle θ as indicated in Fig. 6 which shows both its side view and its elliptical cross-section. The transducer point-spread function is assumed constant throughout the vessel. Since attenuation at any point (x, y) within the tube is due solely to overlying material with thickness d , the signal F for (x, y) is given by the following (A being the unattenuated signal intensity level):

$$F(x, y) = A \left(10^{-0.1(\alpha_f + \alpha_h)d} \right) = A 10^{-0.1\alpha b \sqrt{1 - \frac{y^2}{a^2}} - y} \quad (1)$$

In eqn 1, b is the length of the semi-major axis, α_f and α_h the attenuation (dB/unit length) respectively at the fundamental frequency and harmonic frequencies. The addition of α_f and α_h produces an effective attenuation coefficient α that is observed through the course of this study. For an idealized beam with constant elevational thickness K , the expected signal at each depth y is

$$P(y) = \int_{-K/2}^{K/2} F(x, y) dx \quad (2)$$

The axial and elevational beam widths can be modeled as Gaussian curves with total area 1 (Kallel et al. 1994). The computation of $P(y)$ is illustrated in Fig. 7. The mean *observed* profile through the vessel is the convolution of the axial Gaussian function B_{axial} with the integral of the product of the ideal $F(x, y)$ and the elevational Gaussian function $B_{elevational}$. Therefore, the observed profile $P(y)$ through the vessel is

$$P(y) = B_{axial} * \int_{-\infty}^{\infty} [F(x, y)] (B_{elevational}) dx = B_{axial} * \int_{-\infty}^{\infty} A 10^{-0.1\alpha b \sqrt{1 - \frac{y^2}{a^2}} - y} (B_{elevational}) dx \quad (3)$$

with convolution denoted by $*$. Given an observed profile P , one can fit the profile to parameters A , α , a , B_{axial} , and $B_{elevational}$ with the latter three assumed constant throughout.

Reference vessel position

Lastly, in the normalization process, the value A is then scaled to the value A_{scaled} that it would have been were the vessel located at the axial location of the cortex by a factor S (i.e. $A_{scaled} = SA$). The factor S accounts for changes in signal intensity due to focal gain differences and the differences in time-gain compensation at the two differing axial locations.

Therefore, corrections are finally made to signal intensity measurements to account for variations in contrast signal by normalizing all measurements to A_{scaled} . This normalization estimates the FBV of the cortex. From the FBV of the cortex and the contrast MTT, perfusion is estimated as the value of $r = \text{FBV}/\text{MTT}$.

Application of reference artery contrast model

The renal artery was used as the reference vessel for all measurements. The model was first used to obtain estimates of both the renal artery size (a) and transducer beam widths B_{axial} and $B_{elevational}$, which remained constant throughout the experiment. Second, the model was used to obtain the signal intensity of 100% dilute contrast A at the vessel depth for every acquired image along with the attenuation coefficient α due to the contrast agent within the artery.

The renal artery size, a , and the beam width parameters, B_{axial} and $B_{elevational}$, were estimated as follows. First, the II frames were selected because of the lack of transducer motion during their acquisition and therefore reduced noise from vessel motion in these cases. Vessel profiles $P(y)$ were computed and averaged in the θ direction (as denoted in Fig. 6) over a limited depth range in order to reduce noise. Using a least-squares criterion, each profile was fitted to eqn 3 for all five parameters (A , α , a , B_{axial} , and $B_{elevational}$). For each refill time and flow rate ($n=5$), fits containing outliers in any parameter were rejected using Dixon's criterion (Dixon 1951). The remaining fits were used to calculate the mean and standard deviation of the beam width parameters B_{axial} , and $B_{elevational}$, as well as the vessel size a .

The values of A and α were then estimated by fixing the constant parameters a , B_{axial} , and $B_{elevational}$ as calculated above in eqn 3, and subsequently refitting the profiles. Since the constant parameters physically have only one value, fixing them at their estimates places the emphasis on the relationship between the remaining parameters A and α . Values of A and α were recorded for every refill time, flow rate, and acquisition. The correlation coefficient of A and α was calculated.

For the DT acquisitions, estimates of A and α were made by following the same procedure as above with a , B_{axial} , and $B_{elevational}$ fixed at their previously determined values. Profiles with fitted values of $\alpha < 0$, indicative of a distorted fit, were refit using a least absolute residuals criterion in order to reduce noise effects. Since the time required to pass the transducers over the volume was short compared to expected changes due to the contrast input, each value of A was assumed to apply throughout the acquired volume.

Measurement of scaling factor S

In order to estimate the scaling factor S , images were acquired of a Model 44 Ultrasound Resolution Phantom (Computerized Imaging Reference Systems Inc., Norfolk, VA, USA) at standoff distances equal to that of the kidney cortex and renal artery minus a constant thickness of overlying material. The ratio of the signal intensities S for the two cases was used to correct for the renal artery being located at a different axial depth than the cortex ROI. Using this ratio, values of A_{scaled} were computed from estimated values of A .

Refill curve estimation

Measurements of the kidney cortex after degassing but before introduction of contrast agent indicated that the tissue baseline signal intensity level was essentially constant throughout the cortex at each flow rate. These tissue baseline intensities were subtracted from the mean signal intensities from the cortex ROI at each refill time point and then normalized to values of A_{scaled} . These normalized mean signal intensities were then plotted as a function of refill time for each flow rate and imaging method. Each measured point y_{ij} on the refill curve was assumed normally distributed with an underlying value μ_{ij} (subscripts refer to time t of the j th acquisition). The values of μ_{ij} were then assumed to follow the exponential expression $FBV_j[1 - \exp(-t/MTT)]$, where FBV_j is a random normal variable with mean FBV . Values of FBV_j may vary from run to run due to contrast agent variation between runs. Modeling the

refill in this manner accounts for any correlation between time points within a given acquisition. All measured y_{ij} values were then fit using SAS 9.1.3 (SAS Institute, Inc., Cary, NC, USA) with these assumptions. Estimates of the underlying FBV and MTT were determined along with their standard errors. Due to the relatively long MTTs, some of the measured refill curves may not have achieved saturation and thus fit poorly to an exponential. For these cases, indicated by an estimated correlation coefficient between FBV and MTT of 1, a line of the form $m_f t$ were fit to the data instead. The linear fit was used in these cases to estimate perfusion without knowledge of either FBV or MTT. Fits were performed on a series of transversely oriented image slices from the DT acquisitions for positions of 10, 15, 20, 25, 30, 35, and 40 mm (all measured from the first acquired frame of the volume) with the ROI kept at the same axial and lateral position. The same fitting process was used on the II images acquired at the 20 mm position for comparison.

In addition to the selected transverse slices, a plane oriented in the longitudinal direction was selected from the DT acquired volumes for analysis. Images were reconstructed for a selected axial-elevational plane. Refill curves for an ROI in the kidney cortex with the same size as the transverse ROI were estimated using the model described above. This ROI was at the same axial distance as the transverse case ROI. Estimated parameters were compared with those of the cortex obtained in the transverse direction.

Results

Representative subtraction images in Fig. 8 show the 20 mm transverse slice imaged using low-power II compared with corresponding DT images. The images show the kidney refilling with time, as expected for corresponding delay times. The fast flow images at any given time are brighter than the slow flow images due to the increased flow rate. Brightness variations between II and DT acquisitions are probably due to differences in contrast concentration for the individual acquisitions. Representative images of the reconstructed images, available as a result of the volumetric acquisitions and not otherwise obtainable, of the kidney in the longitudinal direction are shown in Fig. 9. The change in contrast levels from the reconstructed images is less apparent than the change from the transverse cases because the reconstructed images are not subtraction images. In addition, the images are of lower quality than those oriented transversely due to the elevational point-spread function of the imaging transducer being wider than the lateral point-spread function. Finally, slice separation effects are present. The 1 mm slice spacing was selected due to speed limitations of the translation apparatus ($v \leq 6$ mm/s) and maximum trigger rate (~ 6 Hz) of the imaging system (therefore in order to obtain slice spacings < 1 mm, the translation apparatus would have to be slowed, making the acquisition of the shorter duration refill images impossible). In theory, slice separation can be decreased, and the decrease should improve image quality as slice spacing approaches the width of the elevational point-spread function.

Based on the fits of all II frames of the vessel excluding outliers determined using Dixon's criteria as previously described ($n=93$), transducer parameters were estimated (see Table 1). After refitting the profiles with the constant physical parameters (a , B_{axial} , and $B_{elevational}$) held steady, values of A and α were obtained. An example of a vessel profile and its fitted curve is illustrated in Fig. 10. Due to the effects of beam shape and attenuation, note how A is generally not given by the peak of the fitted profile. The correlation coefficient between A and α was determined to be 0.767 ($p < 0.001$) which is consistent with the linear relation with contrast concentration (related to A) and attenuation as reported by Sarkar et al. (2005). The ratio $S = A_{scaled}/A$ accounting for the difference between the upper and lower regions of the kidney due to geometric differences in the beam was found to be 4.7 for the imaging transducer at the specified settings based on the measurement of the contrast phantom. This

value was used to scale all estimated A values to their corresponding A_{scaled} values for the perfusion estimates.

After normalizing all of the raw kidney cortex values by their corresponding A_{scaled} values just described, underlying refill curves were estimated. The refill curves for the 20 mm transverse slice are illustrated in Fig. 11. Combining all the II for the transverse case ($n=5$ for slow flow; $n=4$ for fast flow after the exclusion of one set due to contrast agent anomalies) and DT data ($n=4$ for both flow rates) at the 20 mm position, estimates were made of the perfusion in the kidney cortex.

Depicted in Fig. 12 are the refill curves for the selected longitudinal slice extracted from the DT acquisitions. The exponential fit was used to estimate FBV and MTT when the correlation coefficient between FBV and MTT was less than 1. Otherwise, the linear fit was used to estimate perfusion without separately estimating the FBV and MTT. Points from the fast flow case tend to be dispersed more widely than points from the slow flow case. The similarity of the exponential and linear fits is shown for the DT fast flow cases in each orientation. Linear fits are illustrated in all the other plots. A comparison of measured perfusion values is shown in Fig. 13 for the 20 mm transverse slice. There was no statistical difference between the II and DT results for each flow rate ($p>0.05$ for both rates and both models), with better agreement using the linear model (linear fit DT results were 1.03 ± 0.26 and 1.08 ± 0.37 times II results for respectively slow flow and fast flow). In addition, perfusion estimates of the kidney cortex were obtained at a number of transverse slices from the DT volumetric acquisitions. These estimates, all derived from the linear fit in order to facilitate comparisons, are shown in Fig. 14. The perfusion varies within the cortex at differing slice locations. Changes in the perfusion follow similar trends at both input flow rates to the kidney, with a possible exception at the 25 mm position. The expected perfusion ratio of the fast flow cases to the slow flow cases would be 2, given the 2:1 ratio in infusion rates; however, the measured perfusion ratio of 5.7 ± 2.8 differed significantly ($p=0.013$) from 2. Estimates for the longitudinal slice are shown in Fig. 15. Since the longitudinal slice is composed of portions of the transverse slices, the perfusion values of the longitudinal slice were indeed not statistically different ($p>0.05$ for both slow and fast flow) from the values of the transverse slices, as determined by a Student t-test taking the transverse slice values as a group and comparing them to the longitudinal value.

The comparison between exponential and linear expressions in estimating perfusion for long transit times is depicted in Fig. 16. Given the exponential expression $y(t) = A[1 - \exp(-t/MTT)]$ (Wei et al. 1998), with A denoting the FBV, if the measured points on the refill curve are distributed such that the FBV is well defined, then the linear fit substantially underestimates the perfusion. In contrast, when the points present on the curve only define the initial quasi-linear portion of the exponential expression, then the curve is well-approximated by a straight line of the form $y(t) = mt$. The degree of linearity between the FBV and MTT is given by their correlation coefficient. As the correlation coefficient increases, the apparent FBV and MTT increase along with their estimated errors (details given in appendix B). Were the correlation between FBV and MTT equal to 1, indicating that the measured points lie solely on the quasi-linear portion of the refill curve, an exponential cannot be fit to the data. As previously mentioned, perfusion can still be estimated using the linear fit under these circumstances.

Conclusions and Discussion

We have demonstrated the efficacy of a new technique in contrast refill imaging. The dual beam technique, implemented here with dual transducers, allows the imaging of contrast destruction/refill data in a three-dimensional volume in a reduced time frame, compared to

traditional techniques (2D real-time refill and II taken at one location at a time). The ability of this approach to obtain perfusion measurements similar to those of conventional methods, but from a volume of tissue, suggests this technique is useful in imaging the vasculature of perfused tissue on a three-dimensional basis. A model was introduced that properly normalizes the measured signal intensity levels to a major vessel, accounting for both vessel (Fig. 6) and beam (Fig. 7) geometry. Estimation of the signal intensity corresponding to the contrast suspension $A_{100\%}$ is necessary to determine absolute perfusion; otherwise, perfusion estimates can only be relative, and would depend on the contrast suspensions being constant for all the cases being studied. The normalization to $A_{100\%}$ is suitable only for vessels located near the tissue in question due to the effects of overlying attenuation. Were they located far from each other, the differences in overlying attenuation would invalidate the normalization.

Kraus et al. (1996) have reported absolute perfusion values of the porcine kidney cortex *in vivo* based on thermal diffusion. They noted that for the renal artery infusion rates examined (with a peak rate of 102.1 ± 26.6 mL/min), the cortex perfusion (reported value of 68.1 ± 25 mL/100 g/min) scales linearly with the infusion rate. Their perfusion measurements had standard errors that were essentially constant over the range of infusion rates greater than zero. Assuming a tissue density of 1 g/mL, the perfusion values obtained in this study at the high flow rate (around $0.003 \text{ s}^{-1} = 18 \text{ mL/100 g/min}$ as seen in Fig. 14) are not statistically different ($p=0.43$) from the perfusion value expected (22 mL/100 g/min) from their study after linear scaling to account for the reduced infusion rate. Our infusion at the slow flow rate was less than the minimum non-zero rate measured by thermal diffusion in the study by Kraus et al. (1996), and any comparison to an extrapolated value has limited utility. Nonetheless, the general consistency between the perfusion measurements reported here and those previously reported using thermal diffusion suggests that this approach is viable as a means of obtaining absolute perfusion measurements.

Our acquired data extended to a refill time of 150 s. In retrospect, had the acquisition included longer time points, estimates of the FBV and MTT would be more accurate, and would be possible in more cases. Such long refill times were the result of study limitations, namely the maximum input flow rate to the kidney phantom. As such, these refill times would not be expected *in vivo*. Nevertheless, the estimation of perfusion is useful in its own right, and being able to do so independent of acquiring latter portions of the refill curve may reduce the overall scan time.

We have shown previously that this dual beam method gives expected results in a laminar tube flow (Chen et al. 2007) and now in a preserved kidney phantom. The measured perfusion ratios of the fast flow cases to the slow flow cases differ from the expected value of 2, possibly due to variation within the phantom vasculature with flow rate changes. Nevertheless, the elimination of contrast from a block of tissue whose thickness is much larger than the imaging beam width (as in the DT cases) did not result in statistically significant differences between perfusion measurements from that calculated from clearance at a single position (as in the II cases) at either flow rate. The reconstructed longitudinal images and their associated refill curves yielded results (Fig. 15) that were not statistically different ($p>0.05$) from those of the originally acquired transverse images, despite the fact that contrast clearance was performed in the transverse orientation. This similarity further suggests that the measured perfusion may be independent of the contrast agent volumetric elimination pattern such as that from a 2D array configured to clear and readout the contrast refill with two independent beams. We hypothesize that the independence of the measured perfusion from the contrast clearance pattern arises from the kidney cortex refill being dominated by capillary filling. Since the large vessels that feed the capillary bed fill rapidly after contrast clearance regardless of clearance zone, the subsequent capillary fill is not

affected by differences in clearance zone thickness. In addition, the manner in which a volume is cleared, whether from the transducer used for the II sequence, the DT clearance transducer, or likely by extension from pulses arising from a 2D array, does not affect the fact that contrast was cleared as long as the clearance is completed quickly in comparison to refill in the vascular space of interest. The contrast refill dynamics into the cleared region therefore are not expected to be affected by the manner of contrast clearance. The DT technique was used to demonstrate the effects of volumetric scanning and several possible dual beam methods that could be used to image a three-dimensional volume. The dual-beam techniques should be clinically applicable to living tissues in order to produce a practical means of obtaining three-dimensional absolute contrast perfusion images.

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Appendix A

An estimation of the baseline tissue harmonic signal is possible without the subtraction image, if the contrast agent were stable throughout the course of the refill curve acquisition. The signal intensities would simply be fitted to the exponential expression $y(t) = A[1 - \exp(-t/MTT)] + C$, where C is the non-zero constant tissue harmonic. Normalization to $A_{100\%}$ would be performed after first subtracting C from every point on the curve. If the signal intensities do not extend sufficiently far in time to accurately delineate A , necessitating the use of a linear fit, C would be the y-intercept of the fitted line. However, since the contrast agent was not stable throughout the course of the refill curve acquisitions, a separate estimate of C was necessary, and it was obtained from the baseline values in the II sequence to then compute the subtraction images described.

Appendix B

Covariance plays a significant role in the perfusion estimates described in this paper. Its effects are especially large when the asymptotic limit of the refill curve is not well defined by the available data (i.e. there was insufficient elapsed time to substantially refill the tissue with contrast agent after clearance). When measured points only define the initial quasi-linear portion of the refill curve, a large family of exponential curves can fit the points well. Given this situation, the errors of the parameters A and B (B being the MTT) in the expression $y(t) = A[1 - \exp(-t/B)]$ become large (described in more general terms in Motulsky HJ and Christopoulos A 2003). As the covariance between A and B approaches 1, their estimated errors grow without bound. However, the perfusion is the ratio $r = A/B$ as previously described. The error of r is described by eqn 1 below (Billingsley 1986)

$$\text{var}(r) = \frac{\partial r}{\partial A} \frac{\partial r}{\partial B} \begin{bmatrix} \sigma_A^2 & \sigma_{AB} \\ \sigma_{AB} & \sigma_B^2 \end{bmatrix} \begin{bmatrix} \frac{\partial r}{\partial A} \\ \frac{\partial r}{\partial B} \end{bmatrix} \quad (\text{A-1})$$

where

$\text{var}(r)$ is the variance of r ,

$\partial r / \partial A$ and $\partial r / \partial B$ are the partial derivatives of r with respect to A and B ,

σ_A^2, σ_B^2 are the variances of A and B,
and σ_{AB} is the covariance between A and B.

Expanding eqn A-1 yields

$$\text{var}(r) = \frac{\partial r^2}{\partial A} \sigma_A^2 + 2 \frac{\partial r}{\partial A} \frac{\partial r}{\partial B} \sigma_{AB} + \frac{\partial r^2}{\partial B} \sigma_B^2. \quad (\text{A-2})$$

Note how the first and third terms are always positive. The second term though is negative for positive σ_{AB} given the partial derivatives of r ($\partial r / \partial A = 1/B$ and $\partial r / \partial B = -A/B^2$). The covariance between FBV and MTT was found to be positive during refill curve estimation. A sufficiently large covariance would then cause the variance of the perfusion to be less than that of either the FBV or MTT. This effect from the covariance accounts for the precision of perfusion estimates obtained through an exponential fit in spite of large errors in FBV and MTT estimates. Illustrated in Fig. 17 are the experimental results of estimates of the FBV, MTT, and perfusion $r = \text{FBV}/\text{MTT}$ (all found with an exponential fit) as a function of the correlation coefficient between FBV and MTT. As the correlation coefficient between FBV and MTT increase, estimates of both the FBV and MTT rise along with their error estimates. However, perfusion values remain relatively steady due to the effects of covariance.

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Fig. 1.

DT method schematic. As previously described (Chen et al. 2007), the DT method uses two transducers translated at a constant velocity v , with the clearance transducer translated over the tissue ahead of the imaging one. The clearance transducer clears the volume of interest and the imaging transducer reads the amount of contrast agent that has refilled since contrast clearance. Based on the separation distance s , the refill associated with a specific interval Δt can be measured. Additional delay can be added by pausing the sweep after clearance and before imaging and the two transducers can be replaced by a single volumetric imaging transducer with sufficient control for both clearance and imaging (Reprinted with permission from Chen et al. 2007).

Fig. 2.

Traditional and low-power II. In a traditional II sequence, a series of high-power pulses is applied starting with the tissue being fully perfused and clearing the volume of contrast. After waiting durations I , additional clearance sequences are fired. The signal intensity from the first clearance frame of each sequence corresponds to the level of refill in interval I . Combining the refill levels after various intervals produces a refill curve. Inserting a minimally destructive low power frame immediately prior to the start of the clearance sequences allows the measurement at low power of the contrast agent signal immediately prior to each clearance sequence. These low-power data can then be directly compared to DT measurements of the same volume.

Fig. 3.

Schematic of DT apparatus and experimental setup. The DT apparatus, modified from the apparatus previously reported (Chen et al. 2007), consists of a DT positioning stage driven by translation (M_T) and separation (M_S) motors. The clearance (T_c) and imaging (T_i) transducers are positioned above the phantom being scanned, with the clearance transducer positioned at the edge of the volume of interest. A pump continuously delivers dilute contrast agent from a stirred flask to the phantom.

Fig. 4.

Kidney phantom with transducer orientations. The kidney phantom is perfused through the renal artery that is connected to a pump. All DT scans are acquired by translating the transducers (T_i and T_c refer to the imaging and clearance transducers, respectively) as indicated by the arrows, with the transducers oriented in the transverse orientation. It was performed in the transverse orientation for a selected slice that constitutes a portion of the scanned volume. Reconstructed images in the longitudinal direction, extracted from scans performed using the DT technique, are oriented perpendicular to the transverse orientation as shown.

Fig. 5.

Example of kidney image. Depicted is a transverse image of the kidney phantom with ROIs indicated in both the cortex and renal artery. The renal artery is considered a major vessel that is always filled with contrast. From the signal profile across the vessel in the axial direction, a value A equal to the signal intensity of the contrast suspension can be derived (details in text). This value A can be used to correct for variations with the infused contrast agent suspension.

Fig. 6.

Renal artery normalization model with imaging transducer directions indicated. a) The renal artery is modeled as a cylindrical tube tilted at an angle θ from the horizontal, in the same plane as the transducer. The contrast agent is intercepted by the ultrasound beam as shown. b) The intersection of the beam with the artery is an ellipse with a vessel radius a . From the geometry, the major axis $b = a/\cos \theta$. The distance d_0 from the upper-half of the curve determines the level of attenuation the agent experiences at any point (x_0, y_0) due to overlying contrast, while the unattenuated upper edge has a baseline signal level of A . The integration of the signal levels across the vessel at each depth yields the observed intensity profile (see details in the text).

Fig. 7.

Computation of vessel profile. The intensity profile across the vessel is computed as follows. At every axial position y , the signal intensity $P(y)$ is the integration of all the intensities at y with respect to the elevational direction x . These intensities are weighted by $B_{\text{elevational}}$ prior to integration in order to account for the elevational beam shape. Due to the presence of attenuation from contrast agent, the intensity $F(x,y)$ is non-uniform and is given in the text. An example of a vessel intensity profile is depicted on the right. Note how the presence of attenuation causes the peak of $P(y)$ to shift toward the transducer from the vessel center.

Fig. 8. Representative ultrasound subtraction images obtained from a transverse slice (20 mm position) from preserved porcine kidney with low-power II and the dual-beam technique. The kidney cortex ROI is indicated by the white box. The dashed line indicates the orientation of the longitudinal slice examined with respect to the transverse slice. Note the increased echogenicity as the kidney fills with contrast over time.

Slow rate (17 mL/min)

Fast rate (33 mL/min)

10 mm

Fig. 9.

Representative DT longitudinal images. Analogous to the images in Fig. 8, the kidney is shown to fill with time. The reconstructed images are of lower quality than those directly obtained because the transducer point-spread function in the elevational direction is wider than in the lateral direction. In addition, the transducer spacing affects image quality in the reconstructed images. The longitudinal ROI is indicated by the white box, with the location of the 20 mm transverse slice shown by the dashed line. The locations of the other examined transverse slices from 10 to 40 mm are indicated by markers located beneath the image. Since these images are *not* subtraction images, the change in contrast levels is less apparent than those shown for the transverse cases in Fig. 8.

Sample Profile and Fit

Fig. 10.

Sample vessel intensity profile through renal artery. The signal intensity through the renal artery, oriented as described in the model illustrated in Fig. 6, is plotted with zero being the axial distance of the renal artery ROI closest to the transducer. The fitted curve (described in the text) is used to estimate A for the image with this profile. Note that, due to beam shape and attenuation effects, A is not the peak of the fitted curve.

20 mm transverse slice refill curves

Fig. 11.

Fitted curves for each refill scenario (transverse slice - 20 mm position). Shown are normalized data points for each refill scenario (acquisition method, flow rate) and their fitted curve. Symbols denote individual acquisitions (each individual n) where a contrast suspension was prepared. One sees the similarity between results from the two techniques as well as the difference with the change in flow rate. The 17 mL/min cases along with the 33 mL/min II scenario were fitted with linear fits because acquired data did not extend sufficiently to permit fitting to the exponential equation. The 33 mL/min DT case depicts both its linear fit and fitted exponential.

Longitudinal slice refill curves

Fig. 12.

Fitted refill curves for the reconstructed longitudinal cases. Analogous to Fig. 11, the normalized data points for each flow rate are plotted with their fitted curve. Since the longitudinal images are extracted from the DT imaging, the normalization factors A_{cortex} used to normalize the obtained values are identical to the ones used for the DT transverse cases.

Fig. 13.

Comparison of perfusion estimates for low-power II and the DT technique (20 mm position transverse slice). Slow and fast refer to the renal artery input flow rates of 17 and 33 mL/min. Error bars refer to estimated (± 1 standard deviation) errors in the measurement of the parameters in question. The fast flow DT measurements were fit using both the linear and exponential models. All other measurements were fit using the linear model. The DT perfusion estimates using the linear model appear consistent (slow flow $p=0.81$, fast flow $p=0.68$) for both flow rates with those measured through II. The perfusion estimate from the exponential model fit also was not inconsistent with the II value ($p=0.08$).

Fig. 14.

Kidney cortex perfusion values, estimated using the linear fit in order to facilitate comparisons, for selected transverse slices throughout the imaging volume. The measured perfusion at the fast rate was 5.7 ± 2.8 times that of the corresponding slow rate. The fast rate to slow rate perfusion ratio was statistically different ($p=0.013$) from the expected value of 2. With the possible exception of the 25 mm position, perfusion trends through the volume were consistent with the change in flow rate.

Fig. 15.

Comparison of perfusion of the selected longitudinal slice at both flow rates (slow and fast being respectively 17 and 33 mL/min). The ratio of the fast rate (linear fit) to slow rate perfusion was found to be 5.7 ± 2.2 , which is statistically different ($p < 0.05$) from the expected value of 2.

Approximation of Initial Portion of Refill Curve with a Linear Fit

Fig. 16.

Approximation of the initial portion of the refill curve. Shown are the perfusion estimates found using both an exponential fit and a linear fit approximation for all fast flow cases plotted as a function of their apparent MTTs and estimated correlation coefficients between FBV and MTT from the exponential fit. The horizontal axis denotes the apparent MTT and the correlation coefficient between FBV and MTT. An exponential cannot be fit to the data as the correlation coefficient becomes 1. The horizontal axis is labeled “apparent” MTT because it is strongly suspected that the actual transit time is significantly shorter, with a corresponding reduced FBV (the 796.4 s and 873.4 s MTT cases had physically impossible values of A of 1.49 and 1.19 respectively).

Correlation coefficient effects on parameter estimates

Fig. 17.

Effects of correlation coefficient between FBV and MTT on estimated parameters. Shown are the parameter estimates of the cases where the refill curve was estimated using the exponential expression $y(t) = \text{FBV}[1 - \exp(-t/\text{MTT})]$ plotted as a function of the estimated correlation coefficient between a) FBV and b) MTT. As the coefficient approaches 1, both the estimated parameters and their errors show large increases. c) The perfusion, $r = \text{FBV}/\text{MTT}$, however, does not exhibit this effect due to the effects of covariance on error estimates. d) The relative error (error of parameter/value of parameter) of the FBV and MTT also shows a large increase as the correlation coefficient approaches 1.

Table 1

Transducer parameters for the imaging transducer and renal artery radius a . Values corresponding to estimates made from fitting observed contrast profiles across the renal artery (n=93) are indicated. Both B_{axial} and $B_{elevational}$ are modeled as Gaussian curves, with their full width at half-maximum (FWHM) values indicated. Their values were subsequently fixed at these means and contrast profiles refit in order to obtain estimates of A and α .

	a	B_{axial} (FWHM)	$B_{elevational}$ (FWHM)
Value (mm)	2.9	1.3	6.3
Standard error	0.05	0.10	0.53