Lung perfusion imaging in small animals using 4D micro-CT at heartbeat temporal resolution

Cristian T. Badea,^{a)} Samuel M. Johnston, Ergys Subashi, Yi Qi, Laurence W. Hedlund, and G. Allan Johnson
Center for In Vivo Microscopy, Box 3302, Duke University Medical Center, Durham, North Carolina 27710

(Received 8 May 2009; revised 26 October 2009; accepted for publication 27 October 2009; published 4 December 2009)

Purpose: Quantitative *in vivo* imaging of lung perfusion in rodents can provide critical information for preclinical studies. However, the combined challenges of high temporal and spatial resolution have made routine quantitative perfusion imaging difficult in small animals. The purpose of this work is to demonstrate 4D micro-CT for perfusion imaging in rodents at heartbeat temporal resolution and isotropic spatial resolution.

Methods: We have recently developed a dual tube/detector micro-CT scanner that is well suited to capture first pass kinetics of a bolus of contrast agent used to compute perfusion information. Our approach is based on the paradigm that similar time density curves can be reproduced in a number of consecutive, small volume injections of iodinated contrast agent at a series of different angles. This reproducibility is ensured by the high-level integration of the imaging components of our system with a microinjector, a mechanical ventilator, and monitoring applications. Sampling is controlled through a biological pulse sequence implemented in LABVIEW. Image reconstruction is based on a simultaneous algebraic reconstruction technique implemented on a graphic processor unit. The capabilities of 4D micro-CT imaging are demonstrated in studies on lung perfusion in rats.

Results: We report 4D micro-CT imaging in the rat lung with a heartbeat temporal resolution (approximately 150 ms) and isotropic 3D reconstruction with a voxel size of 88 μm based on sampling using 16 injections of 50 μL each. The total volume of contrast agent injected during the experiments (0.8 mL) was less than 10% of the total blood volume in a rat. This volume was not injected in a single bolus, but in multiple injections separated by at least 2 min interval to allow for clearance and adaptation. We assessed the reproducibility of the time density curves with multiple injections and found that these are very similar. The average time density curves for the first eight and last eight injections are slightly different, i.e., for the last eight injections, both the maximum of the average time density curves and its area under the curve are decreased by 3.8% and 7.2%, respectively, relative to the average time density curves based on the first eight injections. The radiation dose associated with our 4D micro-CT imaging is 0.16 Gy and is therefore in the range of a typical micro-CT dose.

Conclusions: 4D micro-CT-based perfusion imaging demonstrated here has immediate application in a wide range of preclinical studies such as tumor perfusion, angiogenesis, and renal function. Although our imaging system is in many ways unique, we believe that our approach based on the multiple injection paradigm can be used with the newly developed flat-panel slip-ring-based micro-CT to increase their temporal resolution in dynamic perfusion studies. © 2010 American Association of Physicists in Medicine. [DOI: 10.1118/1.3264619]

Key words: x ray, micro-CT, digital subtraction angiography, small animal, lung, perfusion, functional imaging, image reconstruction

I. INTRODUCTION

The heart beats faster in rodents than in humans (six times faster in rats and ten times faster in mice) and imaging the first pass kinetics of a bolus of a contrast agent is challenging with all preclinical imaging modalities, i.e., MR microscopy, microPET, microSPECT, micro-CT, and digital subtraction angiography (DSA). Arterial spin labeling¹ and dynamic contrast enhancement² (DCE) have been used in MR microscopy. Perfusion in rodents has also been measured via nuclear techniques, such as microPET using Oxygen 15-labeled water³ and microSPECT.⁴

Acquisition of perfusion information requires rapid scanning and has become possible with micro-CT by the introduction of slip-ring-based scanners capable of acquiring 3D image data sets once per second for periods of a minute or longer.^{5,6} The reported voxel size in such dynamic scans was 150 μm in plane and 450 μm along the longitudinal axis. For comparison, typical first pass perfusion studies in humans use clinical CT systems with a temporal resolution of 1 s, or roughly 1 image/heartbeat. Depending on the application, the requirements for temporal resolution in human CT perfusion studies can be reduced to every 2–3 s without ad-

versely affecting the perfusion values.⁷ On the other hand, other human studies on cerebral CT perfusion have shown that a 0.5 s temporal resolution would be required.⁸ Scaling the typical temporal resolution of one image per second from a human CT perfusion study to the rodent, with one image every heartbeat requires an interscan time on the order of 150 ms in rats and 100 ms in mice. This requirement is further supported by the fact that not only heart rate but more importantly, the cardiac index, scales in a similar way between mice and humans. The cardiac index in mice is approximately twice that of rats and about ten times greater than in humans.^{9,10} In general, a larger interscan time in small animals would result in missing the peak of enhancement required for accurate perfusion estimation. Therefore, even the fastest micro-CT systems are not fulfilling the requirements for perfusion imaging in rodents. The need for micro-CT perfusion imaging exists. Models of pulmonary disease (for example, emphysema and pulmonary fibrosis) have been studied previously with micro-CT.^{11,12} 4D micro-CT-based perfusion imaging can add important blood flow information. The application in tumor models may be even more important. In many vascular studies, adequate temporal information can be obtained from a 2D projection image. In this case, functional perfusion imaging, and angiographic imaging in general, can be addressed particularly well in small animal models of disease using DSA. We have previously used DSA for both lung and tumor perfusion in the rodents.^{13,14} To overcome the projection nature of DSA, tomosynthesis has been used to recover the depth information.¹⁴ This tomographic DSA (TDSA) approach is based on the paradigm that the same time density curves can be reproduced in a number of consecutive injections of contrast agent at a series of different angles of rotation. A similar multiple injection paradigm was used by Mistry *et al.*¹⁵ with DCE MRI. Since in TDSA we have used a limited angle acquisition and tomosynthesis as a reconstruction algorithm, isotropic resolution was not possible. In the current study, we focused on the challenging application of lung perfusion in rodents by extending our approach to full 4D sets (3D isotropic spatial+time) for perfusion imaging, while maintaining heartbeat temporal resolution.

II. MATERIALS AND METHODS

The challenges of dynamic first pass imaging of perfusion in small animals were addressed by using a dual tube/detector micro-CT system, a microinjector able to deliver multiple precisely controlled injections, and an iterative reconstruction algorithm.

II.A. Imaging system

The dual tube/detector micro-CT system used in this work has been described previously.¹⁶ The system uses two Varian A197 x-ray tubes with dual focal spots ($f_s = \{0.6, 1.0\}$ mm). The tubes are designed for angiographic studies with high instantaneous flux and total heat capacity. Two Epsilon high frequency x-ray generators (EMD Technologies, Quebec, Canada) are used to control the x-ray tubes. The system has

two identical XDI-VHR 2 detectors (Photonic Science, East Sussex, United Kingdom) with a $\text{Gd}_2\text{O}_2\text{S}$ phosphor with pixels of $22\text{ }\mu\text{m}$, 110 mm input taper size, and 4008×2672 image matrix. Both detectors allow on-chip binning, and subarea readout to allow higher speed readout of 10 frames/s, i.e., a time resolution of 100 ms. In order to increase the sampling speed, we have constrained the number of rows read from the detector to the region of the lungs and also binned the pixels 4×4 , i.e., to an effective size of about $88\text{ }\mu\text{m}$. For our system, both tubes and detectors are mounted on a table together with the rotation stage. The animal is positioned vertically in a cradle that is rotated using an Oriel Model 13049 digital stepping motor. We have recently introduced a geometric calibration method for the dual tube/detector system, which allows combinations of projections from the two detectors in a single reconstruction for faster dynamic scanning.¹⁷ Due to our dual imaging chains, projection data have to be acquired only over a 90° +fan angle, rotation. The fan angle for our system is 6.27° .

II.B. Microinjector

The microinjector, an essential component for the perfusion experiments, was developed in-house and tested extensively for our DSA studies.¹⁸ This device consists of a computer-controlled solenoid valve attached to the contrast injection catheter, a heated contrast agent reservoir, and power from compressed N_2 (60 psi). For our protocol, the contrast agent Isovue (Bracco Diagnostics Inc., Princeton, NJ) is heated to the body temperature of the animal (37°C), to eliminate the thermal shock and to reduce contrast viscosity. The high pressure driving the injection compensates for the small caliber lumen and high resistance of the injection catheters (3 Fr) achieving the high flow velocity required for the tightly controlled bolus injections. The microinjector controls the total volume of contrast agent injected via the injection time. This device reproducibly injects volumes down to $6\text{ }\mu\text{L}$.¹⁸

II.C. Sampling strategy

The biological pulse sequence and the sampling strategy are similar to the one proposed for our TDSA approach,¹⁴ but instead of a limited arc acquisition and reconstruction with tomosynthesis, we now acquire projection data over at least 180° +fan angle and use CT reconstruction algorithms. The sampling strategy is presented in Fig. 1. A computer running a LABVIEW (National Instruments, Austin, TX) application controls the timing for the sampling sequence, i.e., the synchronization of all of the events required to reliably reproduce the time density curves required for perfusion imaging. Intubation and ventilation are employed to control the respiratory motion.¹⁹ A perfusion sequence starts at angle 0° by first placing the animal in suspended respiration for approximately 5 s. A series of images is captured on every heartbeat at the QRS complex precontrast (tp_1) and postcontrast injection ($tp_M \geq tp \geq tp_2$). With our dual tube/detector micro-CT system, we can sample two orthogonal projections simultaneously for each time point tp_1 to tp_M over the complete time

FIG. 1. The sampling involves multiple injections, Inj_1 to Inj_N , which create TDCs. Two orthogonal projection images represented here as two orthogonal lines in the Fourier space are acquired at each of the sampling time points tp_1 to tp_M and are synchronized with R peak of the ECG signal. Following each injection and sampling the first pass perfusion, the specimen is rotated by $\Delta\alpha$. Each imaging chain acquires projection data for approximately $90^\circ + \text{fan angle}$ and the two imaging chains collect together projections over $180^\circ + \text{fan angle}$ for each time point. Note that the Fourier distribution of projections is not the same for each time point. A 3D reconstruction is performed for each time point by combining projection images from multiple injections. The temporal resolution is maintained to heartbeat.

density curve (TDC). TDC refers to the change in x-ray opacity over the first pass of the bolus of contrast agent. Based on the central slice theorem,²⁰ the projections are represented as two orthogonal radial lines in the Fourier domain. Note that our radial line representation is only valid for 2D parallel beam projections and only shown here for display purposes (see Fig. 1). In reality, our acquisition uses the cone beam geometry. The animal is rotated to the next angle with an increment $\Delta\alpha^\circ = 6^\circ$ and ventilated for at least 2 min before another perfusion sampling sequence is repeated. The number of injections and the rotational increment are linked so that $N \cdot \Delta\alpha^\circ \cong 96^\circ$.

Based on the assumption of close similarity between the TDC curves obtained with each injection, the projection images fill the Fourier space (see bottom of Fig. 1) and can be assembled into multiple consistent sets for 3D isotropic tomographic reconstruction of perfusion at each heartbeat.

Although represented for simplicity as a single acquisition at tp_1 , in practice, four precontrast injection images are acquired for each injection. A series of MATLAB (The MathWorks, Natick, MA) functions are applied postacquisition to average the four precontrast images and to create a mask. Logarithmic subtraction of the postcontrast and the precontrast mask images provide the DSA-like projections se-

quence containing the vascular/perfusion information [see Fig. 2(A)].

II.D. Multiple injections

As schematically presented by Fig. 1, our perfusion imaging at heartbeat temporal resolution is based on the assumption of close similarity of the TDCs over multiple injections separated in time by at least 2 min intervals to allow for clearance.

We performed experiments to assess the TDC reproducibility in Fischer 344 rats (Charles River, Raleigh, NC). In each study, we used a total of 16 injections of equal volume injected through a jugular vein catheter using our in-house developed microinjector. The imaging sequence was the same as used for the tomographic perfusion studies, but without rotation. Therefore, multiple perfusion sequences at heartbeat resolution were acquired at the same angle. The total volume of contrast agent injected per study was in the range 0.8–1.2 mL. Note that this volume is not delivered in a single bolus, but in multiple small injections over about 32 min.

The heart rate was relatively constant during the experiments, although different from one animal to another. Typical variation in heart rate through the course of the 16 injections was $\sim 5\%$. After acquisition, images were processed to create the DSA sequences and TDCs were computed by measuring at each time point the mean values in the same region of interest (ROI) selected in aorta for all 16 injections. The selected ROI in aorta is not subject to superposition due to the projection nature of DSA. We have derived the mean transit times (MTT) using the singular value decomposition method²¹ implemented in MATLAB. We also compared the average TDC and the derived MTT values of all 16 injections with those corresponding to the first eight and last eight injections. Our goal was to understand if the reproducibility of TDCs is affected when we increase the number of injections from eight to 16.

II.E. Tomographic reconstruction

To minimize possible physiologic complications associated with multiple injections, we aimed to reduce the number of injections used in a study. We were aided by the fact that each injection creates two sets of orthogonal projections with our dual tube/detector system. But even with this advantage, our very limited number of injections (16) results in a limited number of projections (32). For such a situation, an iterative reconstruction algorithm, i.e., the simultaneous algebraic reconstruction technique (SART),²² was preferred to filtered backprojection (FBP) implemented by the algorithm of Feldkamp.²³ Algebraic reconstruction algorithms represent the imaging process as a linear system of equations, in which the task of image reconstruction corresponds to finding the solution of the system of equations. The matrices involved in this approach that we typically use can be prohibitively large, so the system of equations is never written explicitly. Instead, matrix multiplication and inversion is formulated implicitly with a set of forward and inverse operations that are

FIG. 2. A DSA sequence of projections for first 20 consecutive heartbeats following one injection of 75 μ L Isovue 370 in a Fischer 344 rat. A ROI (arrow) was selected in the aorta. The TDCs for ROI in aorta corresponding to 16 consecutive injections are shown in (B). The values corresponding to the MTTs for each injection are shown in (C).

approximations of the true linear operations, and these operations are called in succession to iteratively refine a reconstruction from an initial estimate. In the case of CT, the forward and inverse operations correspond to reprojection and backprojection, respectively, and the initial estimate is typically either the result from the FBP algorithm, or a blank image (all elements set to 0). At each iteration of SART, the reconstruction from the previous iteration is reprojected to generate a set of synthetic projections, these synthetic projections are subtracted from the true projection images acquired during the scan, and the resulting residual images are backprojected on to the reconstructed volume. The residual images may optionally be multiplied by a relaxation factor before updating the volume, but in our studies a relaxation factor of 1 typically provides the best results.

The SART algorithm was implemented on a GeForce 8800 GTX graphics card (NVIDIA, Santa Clara, CA) using the compute unified device architecture (CUDA) general purpose parallel computing architecture (www.nvidia.com/cuda). This technology makes it possible to perform a function simultaneously on multiple processors in parallel using consumer graphics hardware with general purpose programming languages. For our system, we use the GPU to accelerate several stages of the SART algorithm: For the backprojection step, we reconstruct multiple voxels in parallel; for the forward reprojection step, we calculate line integrals for multiple pixels in parallel; and for other arithmetic steps, such as subtracting the reprojected images from the original projections, multiplying the subtracted volume by a relax-

ation factor, and adding the subtracted volume to the reconstructed volume, the arithmetic operations are performed on multiple voxels in parallel. The initial estimate was a zero image.

II.F. Simulations

To investigate the correctness of the SART reconstruction and its robustness to noise and artifacts associated with limited number of DSA projections, we performed simulations using the Moby mouse phantom developed by Segars *et al.*²⁴ This phantom was modeled with nonuniform rational B spline surface based on data collected at the Duke Center for *In Vivo* Microscopy (Durham, NC). The phantom program was run to create a 3D data set (size 256^3 with isotropic voxels of 88 μ m) without respiratory or cardiac motion in order to match one of the time points in our real perfusion experiments involving gated scanning. Since our perfusion application deals with contrast agents, we have also created a modified contrast-enhanced phantom set in which the attenuation values were changed to reflect the use of the contrast agent in our perfusion experiment, i.e., the voxels attributed to blood were increased by an equivalent of 300 HU and those of the lungs by 150 HU. These are typical values in the range of those obtained during a tomographic perfusion study using a bolus injection of contrast agent. Both the original (no contrast agent) and with contrast 3D sets were virtually projected in a cone beam geometry similar to the x-ray micro-CT imaging process using the cone beam pro-

jector implemented in CUDA. In order to provide more realistic estimations, we have also added noise to the projection images to match our experimental noise levels of about 72 HU in a typical *in vivo* mouse micro-CT experiment with Feldkamp-based reconstruction.

Sets of realistic DSA projections were generated by subtracting noisy projections with and without the contrast agent. We note the noise levels are further increased due to subtraction.²⁵ We have used these realistic sets of DSA projections to investigate image quality aspects related to the SART reconstruction algorithms. In this study, we used three sets of 16, 32, and 64 noisy DSA projections with an equiangular distribution over 186° reconstructed using both SART and FBP, i.e., the algorithms of Feldkamp. For a quantitative comparison, we also compared line profiles and computed the root mean square error (RMSE) with the formula $RMSE = \sqrt{1/N_V \sum_{i,j,k} (f(i,j,k) - b(i,j,k))^2}$, where N_V are the total number of voxels in the 3D sets and f and b are the two compared images (the true and reconstructed one).

Iterative reconstruction algorithms such as SART require a decision on the number of iterations needed for convergence, so we ran SART for 50 iterations and computed the RMSE and the percentage RMSE change between successive iterations as figure of merit to assess level of convergence versus number of iterations.

II.G. Animal experiments

All animal studies were approved by the Duke University Institutional Animal Care and Use Committee (IACUC). For lung perfusion studies, we used Fischer 344 rats (Charles River, Raleigh, NC). A 3F catheter was placed in the right jugular vein for contrast agent delivery. The animals were anesthetized with Nembutal (50 mg/kg, IP, Abbott Laboratories, North Chicago, IL) and butorphanol (2 mg/kg, IP, Fort Dodge Animal Health, Fort Dodge, IO), perorally intubated, and mechanically ventilated at 60 breaths per minute with a tidal volume of 2.0–2.2 mL. Following established procedures on our micro-CT system, the rodents were placed in a cradle in a vertical position during imaging. Anesthesia was maintained with isoflurane (1%–2%, Halocarbon Laboratories, River Edge, NJ). Body temperature was measured with a rectal thermistor and maintained at normal levels with a PID feedback-controlled heat lamp. Solid-state transducers on the breathing valve measured airway pressure and flow. Pediatric electrodes were taped on the footpads for ECG. All physiologic signals were continuously collected (Coulbourn Instruments, Allentown, PA) and displayed on a computer using LABVIEW software. These physiologic signals were also used to control the gating described previously in Sec. II C. At the conclusion of the studies, the animals were euthanized with an overdose of anesthesia. For the 4D micro-CT studies, we used 16 injections of 50 μ L each, with a total volume of 0.8 mL. The x-ray settings were 80 kVp, 160 mA, 10 ms/exposure. During a 4D micro-CT perfusion experiment, for each DSA perfusion sequence (acquisition angle), we acquired 20 projections from which four were acquired precontrast injections and used to create a

mask. Therefore, the total number of projections acquired for all 16 injections was 640. The total exposure time was 3.2 s and the total acquisition time in an experiment was approximately 32 min.

Dose was measured using a wireless dosimetry system mobile MOSFET TN-RD-16, SN 63 (Thomson/Nielsen, Ottawa, ON, Canada). Five MOSFET dosimeters silicon chips (1 mm² active area 0.2×0.2 mm²) were positioned at the surface and the center of a rodent like phantom made out of acrylic. The experiments involving the dose measurements have been described previously in Ref. 26.

III. RESULTS

Figure 2(A) presents a typical DSA sequence of projections at heartbeat temporal resolution following an injection of contrast agent in a Fischer 344 rat (weight 163 g with a heart rate of 390 ± 18 beats/min). Figure 2(B) illustrates the reproducibility of the time density curves (TDCs) corresponding to 16 consecutive injections without rotation between runs. These TDCs were measured in similar regions of interest selected in the aorta containing more than 150 pixels. The average TDCs for the first eight and last eight injections are slightly different; for the last eight injections, both the maximum of the average TDC and its area under the curve are decreased by 3.8% and 7.2%, respectively, relative to the average TDC based on the first eight injections. The MTTs of the first eight injections and last eight injections were significantly different ($p < 0.05$). The average MTT values corresponding to the first eight and last eight injections were 1.56 ± 0.03 and 1.52 ± 0.03 s. The difference between the two averages is therefore less than 2.5%.

Since our perfusion application involved a minimum number of injections and therefore a limited number of projections for tomographic reconstruction, we have implemented the iterative SART algorithm. With noisy and limited number of projections, the SART reconstruction starting with a zero was a better choice than when a FBP was used as initial estimate.

Figure 3 shows a comparison between the SART and FBP (i.e., Feldkamp²³) reconstruction of the same axial slice when using 16, 32, and 64 subtracted simulated DSA projections with realistic noise levels. These correspond to eight, 16, and 32 injections with our dual tube/detector micro-CT system. The original true image is also shown. The advantage of SART over the analytical methods is clear, since SART reconstructions appear to be less affected by streaking artifacts and noise. A comparison of line profiles [Fig. 3(H)] shows that indeed SART reconstructions are smoother than the FBP reconstructions and are able to recover the true lung perfusion values. However, the aorta values are not correctly recovered.

Quantitative measures of image quality and convergence were obtained using the RMSE errors.

Figure 4 presents a plot of RMSE [Fig. 4(A)] and the percentage change in RMSE [Fig. 4(B)] for SART with 16, 32, and 64 projections for 50 iterations. Note that all SART reconstructions give a lower RMSE than FBP. The percent-

FIG. 3. The SART reconstruction after 20 iterations using (A) 16, (B) 32, and (C) 64 projections. The equivalent FBP reconstruction is shown in (E)–(G), while the true phantom image is shown in (D). The line profiles show that the SART reconstruction recovered the lung enhancement values well, but was not able to recover the blood vessel. Overall the SART reconstruction with few projections is smoother than the equivalent FBP reconstruction via the Feldkamp algorithm.

FIG. 4. (A) The RMSE computed for SART reconstructions with simulated noisy DSA projections for the Moby Phantom with a weighting factor of 1. Note that all SART reconstruction give a better RMSE than FBP (A). (B) The percentage error change in RMS is less than 3% (around ten iterations) and less than 1% (around 20 iterations).

FIG. 5. The SART-based tomographic reconstruction at iteration 10 of a time point 9 (i.e., after the ninth heartbeat after the bolus injection), data reveal the 3D nature of the perfusion data. The 16 axial slices indicated on a maximum intensity projection image (A) are distanced by 1 mm and are displayed from bottom to top in (B).

age error change in rms is lower than 3% around ten iterations and approximately 1% around 20 iterations [Fig. 4(B)]. These results suggest that in many situations it is acceptable to reduce the number of iterations to only ten in a trade-off between the accuracy and the reconstruction time. The reconstruction time achieved with our implementation for a 256^3 was 0.25 s/projection/iteration.

We also present experimental data from a lung perfusion study in Fischer 344 rats, in which we used 16 injections of $50 \mu\text{L}$ /injection. To show the tomographic nature of our perfusion information, Fig. 5 presents 16 axial slices through the lungs that are separated by 1 mm along the long axis. These SART reconstructions at ten iterations correspond to a single time point (heartbeat, nine postcontrast injection) in a dynamic perfusion sequence. Both the axial slices and the maximum intensity projection [Fig. 5(A)] indicate the ability to distinguish large pulmonary vessels apart from the left ventricle and lung parenchyma. Since it contains some dense contrast agent, the tip of the catheter used for injections appears very bright in these reconstructions.

However, our micro-CT perfusion data are truly 4D (3D isotropic+time). To illustrate the temporal aspect, an axial slice through the dynamic sequence of 16 time points is shown in Fig. 6. SART with 32 projections was used for reconstruction. Although the first image in the sequence is affected by some artifacts caused by the high opacity of the bolus of contrast agent (typical in CT imaging), the sequence displays an image quality that is adequate for quantification of blood flow using the singular value deconvolution (SVD) based approach.²¹ The SVD-based perfusion maps illustrating pulmonary blood flow (PBF), pulmonary blood volume (PBV), and MTT are shown in Fig. 6(B). We have considered the right ventricle as the place for sampling the input function. In our studies, the catheter is placed in the jugular vein and during the injection the bolus goes directly into the right ventricle from where it is pumped into the lungs via the pulmonary arteries. The right ventricle therefore offers a

FIG. 6. (A) An example of 16 successive micro-CT perfusion images in an axial slice cutting through the lung at heartbeat temporal resolution (140 ms). The images were reconstructed at $88\ \mu\text{m}$ voxel size. The SVD-based perfusion maps for PBF, PBV, and MTT are shown in (B). The units for MTT are in seconds, PBV is given in mL and PBF in mL/s.

larger region of interest for the selection of the input function and it was preferred to the pulmonary arteries, which are smaller in size and therefore more sensitive to noise. We note that the perfusion maps are not validated. Further work using fluorescent microspheres would be required to validate these measurements.

Since one of our goals is to reduce the number of injections to a minimum, we have also performed a SART reconstruction using 16 projections corresponding to eight injections per experiment. The slices reconstructed with 32 and 16 projections are compared by Figs. 7(A) and 7(B) together with line profiles in Fig. 7(C). Although noisier, the reconstruction using 16 projections seems to provide very similar values to the 32 projections reconstruction. We have also selected ROIs in the left and right ventricles and lung parenchyma and plotted the mean values of these ROIs, i.e., the time attenuation curves [see Fig. 7(D)]. There is almost a perfect coincidence of these TDCs for the two reconstructions using 16 and 32 projections, which suggests that the number of injections and projections can be reduced. The time required for ten iteration SART reconstruction of a set corresponding to a single time point was 225 s on our GPU-based implementation. Therefore, the total processing time for 16 time points was approximately 1 h. The radiation dose associated with the 640 projections is 0.16 Gy and is therefore in the range of a typical micro-CT dose.²⁷

IV. DISCUSSION

Imaging perfusion in small animals is extremely challenging due to the requirements for high temporal and spatial resolution. This study describes a new imaging method based on 4D micro-CT, which is able to provide both isotropic spatial resolution and the highest temporal resolution (i.e., heartbeat) yet achieved in rodents. Our approach is based on the similarity of TDCs when multiple very small contrast injections are used. Although the TDCs measured in the aorta are not identical [see Fig. 2(B)], their shape and general values are well preserved. A very important question that we aimed to answer was: How many injections can be used while still ensuring a similar TDC? Our tests used 16 injections and we compared the average TDC and MTT values of the first eight and last eight injections and found that these were slightly different.

The factors that could contribute to these small differences may be hypervolemia and vasodilation due to the contrast agent, but their influence is limited based on the small volume injected. The total volume injected for 16 injections in rats is less than 10% of the total blood volume in a rat. This volume was not injected in a single bolus, but in multiple injections separated by at least 2 min interval to allow for clearance and adaptation. We note also that Isovue is a nonionic monomer with low osmolality (iopromide). The lit-

Fig. 7. A comparison between two reconstruction of the same slice with SART using (A) 32 and (B) 16 projections. (C) A plot of a line profile through the two images shows that although noisier, the SART reconstruction using 16 projections is very similar to SART with 16 projections. In (D), TDCs were plotted for mean values in ROIs placed in the lung parenchyma, and the left and right ventricles for reconstructions using SART with 32 and 16 projections. The two sets of curves almost coincide.

erature on the pulmonary hemodynamics effects of different radiographic contrast media indicates that iopromide has been shown to have the least effects on pulmonary vascular resistance of both the normotensive and hypertensive rat lung preparation.²⁸ Consequently, we believe that by carefully controlling the volume of contrast injected and the time between injections we can ensure a relatively reproducible TDC in each animal even if the number of injections is 16. However, a reduction in number of injection is always desirable. As shown by Fig. 7, although noisier, the 16 projections reconstruction seems to provide very similar values to the 32 projections reconstruction. There was almost a perfect coincidence of these time attenuation curves for the two reconstructions using 16 and 32 projections [Fig. 7(D)]. Therefore, we believe that a 16 projection reconstruction is adequate for many applications, especially in the mouse, where the total

volume of contrast agent injected should be further reduced. Work is under way to test the perfusion imaging in mice.

The reconstruction, however, becomes more challenging with fewer injections and therefore fewer projection images. Our selection of the SART algorithm was appropriate when using limited number of projections (see Figs. 2, 5, and 6). As indicated by the line profile plots [Fig. 3(H)], the SART reconstructions are affected by some overall blurring, which is beneficial in the reduction in artifacts due to undersampling. However, the same smoothing effect acts against the recovery of aorta values, which appears as a high frequency structure. Therefore, even if our reconstructed voxels were $88 \mu\text{m}$, the achieved resolution is expected to be lower. Our present efforts to solve this problem are concentrated on developing edge preserving algorithms that are based on a total variation minimization, which we have used for cardiac micro-CT imaging.²⁹ Using the subtracted DSA projections seems to be especially appropriate for such algorithms, since these are sparser than the unsubtracted sets and total variation CT can use this sparseness as a prior. Nevertheless our approach is not limited to the use of subtracted projections.

Due to the design of our instrument, the studies involved scanning rodents in a vertical position. The influence of a vertical body position on murine hemodynamics over time has been investigated with MR microscopy.^{30,31} The conclusion was that tilting the animal to a vertical position introduced no significant change in the animal's hemodynamics. Similar results were shown for rats.³² Thus, the vertical position was not considered to be a major concern in our study.

Although our imaging system is in many ways unique, we believe that the multiple injection paradigm can be used with the newly developed flat-panel slip-ring-based micro-CT systems with 1 s rotation.⁵ A microinjector integrated with imaging and synchronized with the biological signals of the animal, as presented in this work, would allow such an approach to be implemented with these commercial systems. To increase the temporal resolution, as an example, six times (sampling period = 0.166 s), we would use six injections and six different gantry rotations of the slip-ring micro-CT system. The injections would need to be separated in time by 2 min intervals to allow clearance of contrast agent. The key point of the approach would be to synchronize the rotation of the gantry and timing of the injection, so that the distribution of projections corresponding to different injections would be interleaved and to allow a full coverage of at least 180° for each time point. We would divide a sampling arc of at least 180° in a number of arcs of 30° , ($180/6$). The first 30° projection would be acquired from the first rotation, first injection acquisition. The following 30° data would be obtained from the second acquisition (second injection), and so on. In terms of reconstruction, full projections sets are available after each injection. These sets could be used to subtract (via reprojections) any accumulation of contrast agent.

4D perfusion imaging demonstrated here has immediate application in a wide range of preclinical studies. A large number of genetic rodent models already exist for pulmonary diseases such as asthma, emphysema, and pulmonary fibrosis for which the 4D micro-CT perfusion described here can

provide excellent blood flow information. Furthermore, our method could serve for tumor perfusion estimation in small animals. Further engineering developments will need to concentrate on different sampling and reconstruction strategies to reduce both the volume of contrast agent used and radiation dose.

ACKNOWLEDGMENTS

All work was performed at the Duke Center for *In Vivo* Microscopy, an NCRR National Biomedical Technology Research Center (Grant No. P41 RR005959) and NCI Small Animal Imaging Resource Program (Grant No. U24 CA092656), and also supported by Grant No. NCI R21 CA124584. We thank Sally Zimney for editorial assistance.

- ^{a)} Author to whom correspondence should be addressed. Electronic mail: cristian.badea@duke.edu
- ¹ D. L. Thomas, "Arterial spin labeling in small animals: Methods and applications to experimental cerebral ischemia," *J. Magn. Reson. Imaging* **22**, 741–744 (2005).
- ² I. C. Benjaminsen, B. A. Graff, K. G. Brurberg, and E. K. Rofstad, "Assessment of tumor blood perfusion by high-resolution dynamic contrast-enhanced MRI: A preclinical study of human melanoma xenografts," *Magn. Reson. Med.* **52**, 269–276 (2004).
- ³ S. H. Yee, P. A. Jerabek, and P. T. Fox, "Non-invasive quantification of cerebral blood flow for rats by microPET imaging of 150 labeled water: The application of a cardiac time-activity curve for the tracer arterial input function," *Nucl. Med. Commun.* **26**, 903–911 (2005).
- ⁴ A. Constantinesco, P. Choquet, L. Monassier, V. Israel-Jost, and L. Mertz, "Assessment of left ventricular perfusion, volumes, and motion in mice using pinhole gated SPECT," *J. Nucl. Med.* **46**, 1005–1011 (2005).
- ⁵ L. Y. Du, T.-Y. Lee, and D. W. Holdsworth, "Image quality assessment of a pre-clinical flat-panel volumetric micro-CT scanner," *Proc. SPIE* **6142**, 614216.1–614216.8 (2006).
- ⁶ S. Greschus, F. Kiessling, M. P. Lichy, J. Moll, M. M. Mueller, R. Savai, F. Rose, C. Ruppert, A. Gunther, M. Luecke, N. E. Fusenig, W. Semmler, and H. Traupe, "Potential applications of flat-panel volumetric CT in morphologic and functional small animal imaging," *Neoplasia* **7**, 730–740 (2005).
- ⁷ K. Miles, L. M. Eastwood, and M. Konig, *Multidetector Computed Tomography in Cerebrovascular Disease: CT Perfusion Imaging* (Taylor & Francis, London, 2007), pp. 47–55.
- ⁸ A. Kamena, F. Streitparth, C. Grieser, L. Lehmkuhl, B. Jamil, K. Wojtal, J. Rieke, and M. Pech, "Dynamic perfusion CT: Optimizing the temporal resolution for the calculation of perfusion CT parameters in stroke patients," *Eur. J. Radiol.* **64**, 111–118 (2007).
- ⁹ *The Mouse in Biomedical Research, Volume 2, Second Edition: Diseases* (American College of Laboratory Animal Medicine, December 14, 2006), edited by J. G. Fox, S. W. Barthold, M. T. Davisson, C. E. Newcomer, F. W. Quimby, and A. Smith (Academic Press, Elsevier Science and Technology Books, 2007).
- ¹⁰ B. J. Janssen and J. F. Smits, "Autonomic control of blood pressure in mice: Basic physiology and effects of genetic modification," *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* **282**, R1545–R1564 (2002).
- ¹¹ S. Shofer, C. Badea, S. Auerbach, D. A. Schwartz, and G. A. Johnson, "A micro-computed tomography-based method for the measurement of pulmonary compliance in healthy and bleomycin-exposed mice," *Exp. Lung Res.* **33**, 169–183 (2007).
- ¹² A. A. Postnov, K. Meurrens, H. Weiler, D. Van Dyck, H. Xu, P. Terpstra, and N. M. De Clerck, "In vivo assessment of emphysema in mice by high resolution x-ray microtomography," *J. Microsc.* **220**, 70–75 (2005).
- ¹³ M. De Lin, E. Samei, C. Badea, and G. A. Johnson, "Optimized radiographic spectra for small animal digital subtraction angiography," *Med. Phys.* **33**, 4249–4257 (2006).
- ¹⁴ C. T. Badea, L. W. Hedlund, M. De Lin, J. S. Mackel, E. Samei, and G. A. Johnson, "Tomographic digital subtraction angiography for lung perfusion estimation in rodents," *Med. Phys.* **34**, 1546–1555 (2007).
- ¹⁵ N. N. Mistry, J. Pollaro, J. Song, M. De Lin, and G. A. Johnson, "Pulmonary perfusion imaging in the rodent lung using dynamic contrast-enhanced MRI," *Magn. Reson. Med.* **59**, 289–297 (2008).
- ¹⁶ C. Badea, S. Johnston, B. Johnson, M. De Lin, L. W. Hedlund, and G. A. Johnson, "4D micro-CT based perfusion imaging in small animals," Proceedings of the SPIE Medical Imaging Conference, San Diego, CA, 2008, Vol. 7258, p. 72582T (unpublished).
- ¹⁷ S. Johnston, G. A. Johnson, and C. T. Badea, "Geometric calibration for a dual tube/detector micro-CT system," *Med. Phys.* **35**, 1820–1829 (2008).
- ¹⁸ M. Lin, L. Ning, C. T. Badea, N. N. Mistry, Y. Qi, and G. A. Johnson, "A high-precision contrast injector for small animal x-ray digital subtraction angiography," *IEEE Trans. Biomed. Eng.* **55**, 1082–1091 (2008).
- ¹⁹ L. W. Hedlund and G. A. Johnson, "Mechanical ventilation for imaging the small animal lung," *ILAR J.* **43**, 159–174 (2002).
- ²⁰ C. A. Kak and M. Slaney, *Principles of Computerized Tomographic Imaging* (IEEE Press, 1988).
- ²¹ L. Østergaard, R. M. Weisskoff, D. A. Chesler, C. Gyldensted, and B. R. Rosen, "High resolution measurement of cerebral blood flow using intravascular tracer bolus passages: Part I. Mathematical approach and statistical analysis," *Magn. Reson. Med.* **36**, 715–725 (1996).
- ²² K. Mueller and R. Yagel, "Rapid 3-D cone-beam reconstruction with the simultaneous algebraic reconstruction technique (SART) using 2-D texture mapping hardware," *IEEE Trans. Med. Imaging* **19**, 1227–1237 (2000).
- ²³ L. A. Feldkamp, L. C. Davis, and J. W. Kress, "Practical cone-beam algorithm," *J. Opt. Soc. Am.* **1**, 612–619 (1984).
- ²⁴ W. P. Segars, B. M. W. Tsui, E. C. Frey, G. A. Johnson, and S. S. Berr, "Development of a 4-D digital mouse phantom for molecular imaging research," *Mol. Imaging Biol.* **6**, 149–159 (2004).
- ²⁵ M. Lin, C. T. Badea, and G. A. Johnson, "Optimized radiographic spectra for digital subtraction angiography in the mouse," IEEE International Symposium on Biomedical Imaging: From Nano to Macro, Arlington, VA, 2004, Vol. 2, pp. 1412–1415 (unpublished).
- ²⁶ M. Lin, G. Toncheva, G. Nguyen, S. Kim, C. Anderson-Evans, G. A. Johnson, and T. T. Yoshizumi, "Application of MOSFET detectors for dosimetry in small animal radiography using short exposure times," *Radiat. Res.* **170**, 260–263 (2008).
- ²⁷ S. K. Carlson, K. L. Classic, C. E. Bender, and S. J. Russell, "Small animal absorbed radiation dose from serial micro-computed tomography imaging," *Mol. Imaging Biol.* **9**, 78–82 (2007).
- ²⁸ S. K. Morcos, "Review article: Effects of radiographic contrast media on the lung," *Br. J. Radiol.* **76**, 290–295 (2003).
- ²⁹ J. Song, Q. H. Liu, G. A. Johnson, and C. T. Badea, "Sparseness prior based iterative image reconstruction for retrospectively gated cardiac micro-CT," *Med. Phys.* **34**, 4476–4483 (2007).
- ³⁰ F. Wiesmann, S. Neubauer, A. Haase, and L. Hein, "Can we use vertical bore magnetic resonance scanners for murine cardiovascular phenotype characterization? Influence of upright body position on left ventricular hemodynamics in mice," *J. Cardiovasc. Magn. Reson.* **3**, 311–315 (2001).
- ³¹ J. E. Schneider, P. J. Cassidy, C. Lygate, D. J. Tyler, F. Wiesmann, S. M. Grieve, K. Hulbert, K. Clarke, and S. Neubauer, "Fast, high-resolution in vivo cine magnetic resonance imaging in normal and failing mouse hearts on a vertical 11.7 T system," *J. Magn. Reson. Imaging* **18**, 691–701 (2003).
- ³² D. J. Tyler, C. A. Lygate, J. E. Schneider, P. J. Cassidy, S. Neubauer, and K. Clarke, "CINE-MR imaging of the normal and infarcted rat heart using an 11.7 T vertical bore MR system," *J. Cardiovasc. Magn. Reson.* **8**, 327–333 (2006).