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Cardiac and Respiratory-Gated Volumetric Murine Ultrasound

Arvin H. Soepriatna^{1,*}, Frederick W. Damen^{1,*}, Pavlos P. Vlachos^{1,2}, and Craig J. Goergen¹

¹Weldon School of Biomedical Engineering, Purdue University, 206 S. Martin Jischke Dr., West Lafayette, IN 47907, USA

²School of Mechanical Engineering, Purdue University, 585 Purdue Mall, West Lafayette, IN 47907, USA

Abstract

Purpose—Current cardiovascular ultrasound mainly employs planar imaging techniques to assess function and physiology. These techniques rely on geometric assumptions, which are dependent on the imaging plane, susceptible to inter-observer variability, and may be inaccurate when studying complex diseases. Here, we developed a gated volumetric murine ultrasound technique to visualize cardiovascular motion with high spatiotemporal resolution and directly evaluate cardiovascular health.

Methods—Cardiac and respiratory-gated cine loops, acquired at 1000 frames-per-second from sequential positions, were temporally registered to generate a four-dimensional (4D) dataset. We applied this technique to 1) evaluate left ventricular (LV) function from both healthy mice and mice with myocardial infarction and 2) characterize aortic wall strain of angiotensin II-induced dissecting abdominal aortic aneurysms in apolipoprotein E-deficient mice.

Results—Combined imaging and processing times for the 4D technique was approximately 2 to 4 times longer than conventional 2D approaches, but substantially more data is collected with 4D ultrasound and further optimization can be implemented to reduce imaging times. Direct volumetric measurements of 4D cardiac data aligned closely with those obtained from MRI, contrary to conventional methods, which were sensitive to transducer alignment, leading to overestimation or underestimation of estimated LV parameters in infarcted hearts. Green-Lagrange circumferential strain analysis revealed higher strain values proximal and distal to the aneurysm than within the aneurysmal region, consistent with published reports.

Conclusion—By eliminating the need for geometrical assumptions, the presented 4D technique can be used to more accurately evaluate cardiac function and aortic pulsatility. Furthermore, this technique allows for the visualization of regional differences that may be overlooked with conventional 2D approaches.

Corresponding Author: Craig J. Goergen, cgoergen@purdue.edu, 206 S. Martin Jischke Drive, West Lafayette, IN 47907, Phone: (765) 494-1517.

*Equal contributions

Conflict of Interest

The authors have no conflict of interest to report.

Ethical Approval

All procedures performed in studies involving animals were in accordance with the ethical standards of the Purdue Animal Care and Use Committee.

Keywords

Animal models and imaging; circumferential strain; abdominal aortic aneurysm; aortic dissection; myocardial infarction; ultrasound; volumetric imaging

Introduction

Cardiovascular disease (CVD) is the leading cause of death in the United States, responsible for 30% of all deaths in 2013 [1]. With CVD expected to affect 40% of the U.S. population by 2030 [2], there is an increasing need to develop improved *in vivo* imaging techniques to better understand the pathophysiology and progression of CVD. Four-dimensional (4D) imaging, also known as gated three-dimensional imaging, has sparked interest in recent years as it delivers a cinematographic representation of three-dimensional structures with high spatiotemporal resolution [3]. The use of time-dependent 3D geometries is becoming more popular as it provides patient-specific boundary conditions for biomechanical modeling and computational fluid dynamic simulations [4]. Recent developments of clinical 4D CT [5], MRI [6,7], and ultrasound [8,9] have enabled the study of valve leaflet motion [10], 3D myocardial strain [11], and aortic wall kinematics [12], overcoming the limitations of standard 2D and 3D imaging techniques. These traditional imaging methods often fail to adequately identify differences in motion associated with a variety of pathological conditions. As a result, the advantage of 4D imaging stems from its ability to capture a holistic view of tissue motion, making it a method of increasing interest for the study of CVD.

Small animal models have proven useful in studying various CVD mechanisms. In particular, the development of *in vivo* imaging techniques for mouse models have become invaluable for controlled investigations of CVD progression, while simultaneously reducing the necessary number of animals per study [13]. Still, there are notable obstacles unique to cardiovascular imaging in rodents that make these techniques challenging. The animals' small size, fast respiration, and rapid heart rate – which ranges from 400 to 600 beats per minute [14] – require an imaging modality with spatiotemporal resolution much higher than that used for humans. While volumetric ultrasound probes exist for large animals and clinical use [15], their low spatiotemporal resolution renders them inadequate for imaging small animals. Recent advancements in high-frequency ultrasound probes have overcome limitations in spatiotemporal resolution by providing a sampling rate adequate for rodents, yet these improvements currently only apply to planar imaging. In these regards, post-processing methods are needed to reconstruct and visualize 4D ultrasound data.

Gating using electrocardiogram (ECG) and respiration signals are often performed in tandem with ultrasound imaging to temporally synchronize periodic events and minimize motion artifacts [16,14]. This paradigm creates a foundation to spatiotemporally combine data from multiple acquisitions, which extends to techniques such as 4D MR microscopy and micro-CT [17,7]. However, as the sampling rate is much slower than that of high-frequency ultrasound probes, these techniques are limited to resolving only large-scale heart motion and often require prolonged administration of anesthesia; in the case of micro-CT,

ionizing radiation and an intravenous contrast agent are also needed. While high-frequency ultrasound imaging also requires anesthesia and is subject to imaging artifacts, its ability to image in real-time makes it ideal for measuring cardiac function and pulsatile vascular motion in mice [18].

To date, the majority of cardiovascular imaging research in rodent models has utilized conventional 2D ultrasound to evaluate left ventricular (LV) function and characterize aneurysm growth. However, the extension to gated volumetric ultrasound provides several advantages over these planar techniques. In addition to eliminating the need for geometric assumptions, which are necessary with 2D imaging [19], 4D imaging is capable of capturing regional differences in ventricular and arterial motion that may go undetected with planar approaches. Here, we demonstrate a method for acquiring and analyzing 4D ultrasound data in small animal models of CVD and compared the results to standard 2D techniques and MRI. To the best of our knowledge, this is the first published report comparing 4D ultrasound, MRI, and 2D echocardiography in small animals, highlighting the limitations of 2D techniques. The proposed method combines a linearly translating high frequency ultrasound transducer, ECG and respiratory gating, and image post-processing to reconstruct 4D data for the direct evaluation of LV health and characterization of dissecting abdominal aortic aneurysm (AAA) wall kinematics.

Materials and Methods

Animals

All mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and were divided into three different experimental groups. The first group consisted of 11 male C57BL/6J wild-type mice and was imaged to evaluate LV function in healthy mice. Mice with ages ranging from 21 to 70 weeks were selected to allow for imaging collection from a wide variety of mouse sizes, but our analysis revealed no statistical correlation between animal age and cardiovascular metrics in these adult mice (data not shown). The second group consisted of 6 male C57BL/6J wild-type mice with varying degrees of myocardial infarctions (MIs) and was imaged four weeks post-MI induction to assess for LV function in remodeled hearts. In preparation for each myocardial infarction surgery, mice were anesthetized with 2% isoflurane, endotracheally intubated, and ventilated with O₂ (SomnoSuite, Kent Scientific, Torrington, CT, USA) [20]. Pneumothorax was avoided by supplying air to the lungs with a target inspiratory pressure between 16–18 cm H₂O and a minimum peak-end expiratory pressure between 3–5 cm H₂O. After performing a left thoracotomy, we permanently ligated the left anterior descending (LAD) coronary artery using 8-0 Nylon sutures [21,22] and allowed the mouse to recover (Online Resource 1a). The third group consisted of 7 male apolipoprotein-E deficient (apoE^{-/-}) mice with dissecting AAAs imaged two weeks post-aneurysm formation to characterize aneurysm wall kinematics. To induce an aneurysm, apoE^{-/-} mice were implanted subcutaneously with mini-osmotic pumps (ALZET Model 2004; DURECT Corporation, Cupertino, CA, USA) to systemically deliver 1000 ng/kg/min of angiotensin II (A9525; Sigma-Aldrich, St. Louis, MO, USA) for 28 days [23,24] (Online Resource 1c). A summary of the characteristics for the different experimental mouse groups is shown in Table 1.

Time-Resolved Volumetric Ultrasound Imaging

Cardiac- and respiratory-gated volumetric ultrasound (4D) data was acquired using the Vevo2100 small animal ultrasound system (FUJIFILM VisualSonics Inc., Toronto, Ontario, Canada) and a 40 MHz linear array ultrasound transducer (MS550D) attached to a linearly translating step motor (3D Acquisition Motor). Before imaging, we anesthetized the mouse with 2–3% isoflurane at 1.5 L/min room air using a low-flow integrated digital anesthetic vaporizer (Somnosuite, Kent Scientific, Torrington, CT, USA). The SomnoSuite more efficiently delivers isoflurane to the animal, which has been shown to effectively minimize heart rate variations during imaging as long as two hours [20]. We removed hair from the region of interest (i.e. the ventral thorax for cardiac imaging or the abdomen for dissecting AAA imaging) using a depilatory cream and applied ophthalmic ointment to the eyes to prevent corneal desiccation. ECG and respiratory signals were continuously acquired by securing the animal's paws to gold-plated leads using an electrode gel while the animal was positioned supine on a heat-modulated imaging stage (Vevo Imaging Station). Respiratory information was obtained by monitoring for low frequency changes in the signal associated with fluctuating electrical impedance during inspiration. We implemented cardiac and respiratory gating by acquiring ultrasound data at successive 1 ms delays between RR peaks and by using a 40% acquisition window in between inhalation peaks. Isoflurane levels were gradually decreased throughout image acquisition to further minimize fluctuations in heart rates. To prevent hypothermia, the animal's body temperature was monitored using a rectal temperature probe, and the temperature of the heated stage was adjusted accordingly. We also used a heating lamp as a secondary source to help maintain body temperature during imaging. On average, setting the temperature of the stage between 40–42°C maintained the animal's body temperature to $36.1 \pm 0.6^\circ\text{C}$, even in experiments as long as 90 min.

Once the respiration rate of the mouse had stabilized, we applied warmed ultrasound gel to the skin and positioned the ultrasound probe at the center of the region of interest, perpendicular to the intended axis of translation. For example, when imaging the LV, short-axis cine loops were collected with the probe translating along the long-axis of the heart. A custom MATLAB script (MathWorks, Natick, MA, USA) was used to automate the translation of the probe and acquisition of cine data at each sequential location. Step sizes ranging between 80 to 200 μm were used for both cardiac and dissecting AAA acquisitions, depending on the length of the region of interest. Cine loops were acquired at 1000 frames-per-second (fps) using the ECG-gated kilohertz visualization (EKV) module (FUJIFILM VisualSonics Inc.). EKV acquisition utilized both cardiac and respiratory gating to reconstruct ultrasound images over one representative cardiac cycle from data over multiple beats. This is only possible because of similarities between sequential heartbeats. As such, each EKV cine loop consisted of planar snapshots of the region imaged across one representative cardiac cycle with a temporal resolution of approximately 1 ms. The number of frames obtained in an EKV loop was dependent on the animal's heart rate, which remained relatively stable throughout image acquisition. Total acquisition times ranged from 40 to 90 minutes, depending primarily on the length of the scanned region and the defined step size, with each slice taking approximately 30–45 seconds to acquire.

Data Reconstruction

The series of position-dependent EKV cine loops were exported to MATLAB for compilation and analysis. Spatially adjacent cine loops were aligned and temporally matched such that each reconstructed volume represents one synchronized time-point in the cardiac cycle. The total number of frames in each successive EKV loop could vary due to heart rate fluctuations. These discrepancies are typically confined to 2–3% of the mean number of frames and were therefore considered negligible. During reconstruction, this was accounted for by removing end-diastolic frames in longer loops before temporally matching the remaining frames. Gaussian filters were applied both spatially and temporally for noise removal; since the voxels were anisotropic, the kernel window used was 10% of the total number of pixels in each spatial dimension, and the Gaussian filter width was chosen relative to a desired isotropic resolution size. Linear interpolation was used to reconstruct isotropic voxels and simplify post-processing analysis: 60 μm for cardiac data and 40 μm for aortic aneurysm data. A smaller voxel size was chosen for the aortic data to better capture the wall boundaries and visualize motion of the stiff dissecting aneurysm. A diagram of the acquisition method is outlined in Fig. 1 using a sample murine cardiac dataset. The MATLAB codes for image acquisition and reconstruction are provided as supplementary materials.

Analysis of 4D Cardiac Dataset

Manual segmentation was performed on the reconstructed 4D cardiac data to outline the endocardial and epicardial surfaces of the LV, extending from the apex to the mitral and aortic valves at end-diastole and peak-systole. To study the contribution of papillary muscle on lumen volume measurements, two endocardial boundaries were obtained: one with the inclusion and a second with the exclusion, of the papillary muscles during segmentation. The segmented boundaries were then used to create 3D representations of the LV at both end-diastole and peak-systole. All volume measurements were converted to physical units by multiplying the number of voxels within the 3D volumes by the physical dimensions of each isotropic voxel. End-diastolic volume (EDV) and peak-systolic volume (PSV) were used to evaluate LV function including stroke volume (SV), ejection fraction (EF), and cardiac output (CO). These measurements were calculated using the following equations (1–3):

$$\text{SV} = \text{EDV} - \text{PSV} \quad (1)$$

$$\text{EF} = \text{SV} / \text{EDV} \times 100 \quad (2)$$

$$\text{CO} = \text{SV} \times \text{heart rate} \quad (3)$$

We also calculated LV volumes using two established methods commonly used in the clinic – the Teicholz method (4) and the modified Simpson's rule of discs (5) – and compared them to LV volumes obtained by direct measurements from the reconstructed 4D datasets.

As recommended by the guidelines for cardiac chamber quantification provided by the American Society of Echocardiography, the papillary muscles were considered part of the LV cavity when performing endocardial wall tracings [25–28].

$$\text{LV Volume}_{\text{Teicholz}} = \frac{7}{(2.4 + d_i)} d_i^3 \quad (4)$$

$$\text{LV Volume}_{\text{Simpson's}} = \frac{\pi}{4} h \sum d_i^2 \quad (5)$$

In (4–5) above, d_i corresponds to the LV internal diameter, and h symbolizes the height of the circular discs.

Analysis of 4D Dissecting AAA Dataset

Similar to the cardiac analysis, the aortic walls of the 4D dissecting AAA datasets were manually segmented at systole and diastole. Branching vessels, including the superior mesenteric artery, celiac artery, and renal arteries were excluded for simplicity. To quantify aortic wall kinematics, maximum Green-Lagrange circumferential cyclic strain ($\varepsilon_{\theta\theta}$) was calculated at each axial location by using the effective systolic (d_S) and diastolic (d_D) diameters calculated from the segmented cross-sectional areas of the abdominal aorta [29]:

$$\varepsilon_{\theta\theta} = \frac{1}{2} \left[\left(\frac{d_S}{d_D} \right)^2 - 1 \right] \times 100 \quad (6)$$

Magnetic Resonance Imaging

MRI data for infarcted hearts were acquired using the Bruker 7T Biospec 70/30 USR System (Bruker, Billerica, MA, USA) and a small animal volume coil (RF RES 300 1H 075/040 quadrature surface transmit receive coil, Bruker). We anesthetized the mouse with 2–3% isoflurane in room air and acquired ECG, respiration, and temperature data using the MR-Compatible Monitoring and Gating System (SA Instruments, Stony Brook, NY, USA). ECG and respiratory-gated cine fast low angle shot (FLASH) pulse sequence (TE = 3 ms; TR = 7 ms; FA = 20°; Matrix Size = 256 × 256; Number of averages = 1) was used to acquire contiguous 1 mm thick short-axis cine loops of a beating left ventricle.

Results

Assessment of Left Ventricular Function

Representative diastolic images of a healthy LV (top) and a LV with a four-week old anterior apical infarct (bottom) are shown in three-axis views in Fig. 2, with ultrasound and MRI cine loop data shown in Online Resource 2 and 3 respectively. The displayed data were rotated along its anterior-posterior axis to visualize true short-axis and long-axis views of the LV.

Due to image registration, volumetric data can be spatially rotated; hence, any planar cut can be extracted to give both conventional (axial and sagittal) and unconventional (coronal) ultrasound views of the heart. Manually segmented 3D renderings of both the endocardial (red) and epicardial (blue) LV walls are illustrated in Fig. 3 for both healthy and infarcted hearts at end-diastole and peak-systole. The asymmetrical anterior apical infarct is associated with wall thinning and dyskinesia of the necrotic myocardium at peak-systole. The unique geometries of all healthy and infarcted LVs at end-diastole and at peak-systole are summarized in Online Resource 4 and 5 respectively. The inclusion of papillary muscles as part of the LV lumen resulted to increases in LV volumes at end-diastole (7.6 μL) and at peak-systole (2.4 μL), contributing to an increase in ejection fraction values (3.11%) as shown in Online Resource 6.

Bland-Altman percent difference analyses (Online Resource 7) and correlation (Online Resource 8) of cardiac parameters calculated using different techniques were conducted for both healthy and infarcted left ventricles. Although conventional 2D techniques performed similarly (within $\pm 10\%$) to the direct 4D method in quantifying EF in healthy LVs, they performed significantly worse compared to both gold-standard MRI and the 4D method when evaluating EFs in infarcted LVs. Box-and-whisker plots showing percent difference for the different cardiac parameters between the 4D, 2D Simpson's, and 2D Teicholz techniques relative to MRI for infarcted LVs are illustrated in Fig. 4(a–e). Table 2 shows the medians, as well as the 1st and 3rd quartile values [Q_1 , Q_3] for the cardiac parameters calculated using the different techniques. Values of EF measured using the 2D Simpson's and Teicholz techniques deviated from that measured with MRI by 38.5% and 30.2% respectively. On the contrary, EF values obtained directly with the 4D technique only differed from that of MRI by 2.3%, with the 1st and 3rd quartile values confined between -10.9% and 17.3% .

Quantification of Dissecting AAA Regional Strain

A three-axis view of a mouse dissecting AAA acquired at diastole two-weeks post aneurysm formation is displayed in Fig. 5. The stratification of the aneurysm into the true and false lumen, as well as the formation of an intramural thrombus (black arrows), can be clearly seen in both the long and short-axis views. 3D masks of the dissecting AAA represent the vessel geometries during diastole (red) and systole (blue) and are shown in Fig. 6a. Increased wall stiffness, caused by the remodeling of the aneurysmal wall, accounted for the small distensibility observed within the aneurysm between diastole and systole [30–32]. Sections of the abdominal aorta proximal and distal to the aneurysm exhibited greater wall displacements as compared to those located in the middle of the aneurysms and its neighboring regions. Quantitative assessment of Green-Lagrange circumferential strain along the abdominal aortic wall is shown in Fig. 6(b–c) and Online Resource 9. The averaged circumferential strains of all seven mice plotted along normalized abdominal aorta positions illustrated that both proximal and distal regions have higher maximum strain values compared to regions within the aneurysm. Although wide variances in maximum strain persisted in proximal and distal regions, aneurysmal regions exhibited relatively low strain values of 1–2% with minimal variance. Online Resource 10 further demonstrates the wall kinematics of a dissecting AAA two weeks following aneurysm formation.

Discussion

In this study, we presented a small animal ultrasound imaging technique to acquire gated volumetric datasets from both cardiac and vascular regions of interest. By employing cardiac-gating, respiration-gating, and image registration in conjunction with a high-frequency ultrasound transducer, we were able to collect volumetric data with both high spatial and temporal resolution. We used our 4D imaging technique to demonstrate that conventional echocardiographic methods perform unreliably in evaluating LV function in mice with myocardial infarction compared to direct volumetric assessment of LV function. We believe this is the first published report quantifying 4D echocardiography in small animals, revealing a better agreement with MRI data than standard 2D ultrasound. We also used our technique to show that Green-Lagrange circumferential strain decreased significantly within the aneurysmal region when compared to regions proximal and distal to the aneurysm. These results suggest that a 4D imaging approach may be advantageous when assessing cardiac and vascular function with complex geometries and kinematics.

Conventional Echocardiographic Techniques Perform Unreliably in Evaluating Function in Diseased LVs

Conventional echocardiographic methods for evaluating cardiac health rely on geometrical assumptions from 2D B-mode slices to approximate LV volumes [26]. These techniques assume that LV cross-sections adopt circular shapes and can therefore be modeled as stacks of elliptical discs. Unfortunately, these 2D techniques are susceptible to issues related to structural abnormalities not captured within the imaging plane, apical foreshortening, and large inter-observer variability [33,27]. Furthermore, these heuristic approaches are often violated when imaging patients or animals with complex cardiac pathologies that have regional dyssynchrony and ventricular dysfunction, leading to inaccurate estimations of LV volumes and ejection fractions [25,34,26]. Using the proposed 4D ultrasound technique described here, we can measure LV volumes and other parameters of LV function directly from complex 3D geometries, addressing these shortcomings.

Even in healthy hearts, the assumptions accompanying Simpson's rule may not accurately reflect the structure of the LV, due to the contribution of papillary muscles, as illustrated in Figs. 2–3 and Online Resource 4–6. These assumptions prove to be especially problematic in the assessment of disease states, such as in hearts with asymmetrical apical infarcts (Figs. 2–3 and Online Resource 5). Depending on the transducer alignment relative to infarct geometry, 2D approximations of LV function may be grossly overestimated or underestimated, as reflected by the large observed percent difference between their values and that obtained from MRI (Fig. 4, Online Resource 7, and Table 2), which we used as a gold-standard for this study. Comparisons of direct 4D ultrasound measurements with MRI, however, demonstrated that the 4D method performed similarly to MRI, as observed by the small percent difference depicted in Fig. 4(a–e). Furthermore, the interquartile ranges for all cardiac parameters obtained using the 4D approach were considerably narrower than those acquired from 2D Simpson's and Teicholz techniques, suggesting that the 4D method performed consistently compared to conventional techniques. Secondly, to compensate for dyskinesia in necrotic myocardial tissue and reduced contractility in regions neighboring the

infarct zone, healthy regions of the left ventricle exhibit increased inotropy. This leads to LV geometries that depart from the assumed elliptical shape used in conventional 2D techniques. The data included here highlight the limitations of 2D ultrasound imaging, suggesting that gated volumetric approaches should be considered in studies of complex cardiac disease models with regional differences. It is important to note that the presented results were obtained from mice with large and asymmetrical infarcts. Further work will focus on studying how the performance of 2D techniques compares to the proposed method for less severe infarction models, such as the ischemia-reperfusion model [35], or in cases where cardiac remodeling has a uniform effect on heart structure. Taken together, the major advantage of the presented 4D technique is its ability to capture exact LV geometry throughout the cardiac cycle, providing a more direct approach when evaluating LV function. This approach also opens the possibility of directly quantifying ventricular synchronicity and 3D strain maps of murine hearts using ultrasound.

Aneurysm Formation Leads to Reductions of Green-Lagrange Circumferential Strain

The 4D ultrasound technique was also used to study regional differences in aortic wall kinematics. Although numerous ultrasound studies on murine dissecting AAAs have been conducted, many still rely on linear measurements of aortic diameter to characterize disease severity and quantify aortic wall strain [36,37]. Variations in aortic wall thickness, influenced by dissections and the presence of intramural thrombus (Fig. 5), may lead to non-uniform distensibility and radial expansion of the aortic wall. In fact, performance comparison between planar-based ultrasound methods showed that the choice of imaging plane significantly affects the accuracy of the parameters such as aortic diameter and stiffness [38]. Using our 4D imaging technique, we were able to mitigate this problem by visualizing the pulsations of the AAA in 3D (Online Resource 10) and characterizing circumferential aortic wall strain by measuring cross-sectional areas of the aorta. Fig. 6(a–c) demonstrates that regions proximal and distal to the aneurysm exhibit high strain values as compared to the aneurysmal region, with the proximal regions showing higher strain values than the distal regions. The presence of branching vessels in distal regions of the abdominal aorta may contribute to the observed decreased strain values. This decrease in maximum Green-Lagrange circumferential strain in the aneurysmal region is likely due to an increase in vessel stiffness. The results described here are consistent with previous reports showing an increase in vessel stiffness due to the degradation of elastin and increased collagen turnover [39,32]. This increase in vessel stiffness was also supported by previous reports demonstrating reductions in pulse-wave velocities along the aneurysmal wall [40–42]. Previous work using 2D M-mode ultrasound suggested that aneurysm formation typically decreased the Green-Lagrange circumferential strain from around 15% to 3% [32]. However, strain analysis using other imaging modalities, such as MRI, documented higher values of circumferential cyclic strain of 20% in healthy suprarenal aorta and 10% in AAAs [43,39]. This discrepancy is potentially due to inherent differences in imaging modalities since time-of-flight MRI has difficulty accurately assessing lumen size in regions of slow or complex flow that are often observed in dissecting AAAs. Furthermore, MRI is subject to lower spatial and temporal resolution compared to ultrasound. Using our 4D technique, we have extended the analysis of Green-Lagrange circumferential strain from conventional 2D M-mode to diameters approximated from cross-sections of the 4D aneurysm data to take into

account for non-uniform distensibility and radial expansion of the aortic wall. Applying a gated volumetric imaging technique to expand pulse wave quantification to 3D could further improve the characterization of mechanical factors that lead to aneurysm initiation, growth, and rupture.

Tradeoff Between Resolution, Acquisition Time, and Processing Time

One important concept to consider when acquiring 4D data is the tradeoff between image resolution and acquisition time. Depending on the region of interest location, size, and the disease model studied, the step size can be carefully selected to minimize acquisition and processing time while obtaining sufficient spatiotemporal resolution. In the presented work, we acquired data using small step sizes ranging from 80 μm to 200 μm and a sampling rate of 1,000 fps so that we were able to easily identify regional differences in the contractility of infarcted LVs and pulsatility of dissecting aneurysms. As such, the average imaging time ranged between 40 to 90 minutes for the cardiac and aneurysm data, respectively, with image reconstruction and analysis taking an additional 30 minutes. Although conventional 2D techniques can take on the order of 30 minutes for both acquisition and analysis combined, the need to obtain multiple 2D views at different positions when studying diseases exhibiting regional differences may significantly increase image acquisition times. Taken together, the results of this study suggest that acquiring 4D data with small step sizes and high sampling rates may not be necessary when assessing global cardiovascular function in healthy mice. The presented volumetric technique, however, allows for the visualization of regional differences that may be overlooked with conventional 2D approaches. Furthermore, the presented ultrasound technique opens the possibility of mapping 3D strain, quantifying ventricular synchrony/dyssynchrony, and estimating vascular pulse wave velocity with high spatiotemporal resolution.

Limitations and Future Work

A small animal ultrasound system capable of collecting 4D data has recently been made commercially available (Vevo3100, FUJIFILM VisualSonics Inc. [44]). This newer imaging platform uses the same basic image acquisition process but has integrated software to automatically acquire 4D data, a maximum temporal resolution of 300 fps, and uses a dual-gating technology to accelerate image acquisition time. Still, the major factors influencing total imaging time are the choice of temporal resolution and step size. In this study, we chose a sampling rate of 1000 fps with small step sizes to allow for the collection of 4D data with both higher spatial and temporal resolution while acknowledging the tradeoff with longer acquisition times.

A limitation of the presented technique is the need to acquire data with minimal variations in heart rates. If large variations in heart rates are observed, temporal resampling and interpolation techniques should be implemented to ensure that images from successive spatial positions match in their cardiac cycle. Within an imaging session, data acquired when the mouse has a lower heart rate will have more time points representing a single cardiac cycle and may therefore need to be downsampled. 4D ultrasound imaging also faces several challenges in regards to data size and processing time. Our image data sizes reached magnitudes of 2–4 gigabytes depending on the dimensions of the structure of interest and

step size between adjacent slices. While post-processing resampling of the compiled original resolution data could mitigate this problem, it sacrifices finer detail and requires data interpolation that may affect overall image quality. 4D imaging is also inhibited by long processing time primarily due to the manual segmentation process. Future studies into more robust and automated wall-tracking algorithms could reduce the segmentation time and improve its use of 4D imaging for other applications. Other future work will be needed to use 4D ultrasound data for computational fluid dynamic simulations and biomechanical modeling of blood flow.

Conclusion

The presented 4D ultrasound technique employs both cardiac and respiration gating to visualize the motion of three-dimensional structures with high spatiotemporal resolution, allowing for the study of complex cardiovascular kinematics in small animals. Specifically, we demonstrated the technique's ability to directly evaluate LV function and characterize Green-Lagrange circumferential strain in murine AAAs. Direct volumetric measurements of the LV from 4D cardiac data matched closely with gold-standard MRI results whereas 2D ultrasound techniques are sensitive to transducer alignment relative to infarct geometry. Strain analyses of murine AAAs demonstrate that the aneurysmal region exhibited lower strain values when compared to proximal and distal sections, likely due to the remodeling of the extracellular matrix within the aortic wall. Given the current efforts in the field focused on studying regional differences in wall motion of mouse models with myocardial infarction and AAA, a 4D ultrasound approach could be beneficial for a wide variety of studies where direct volumetric measurements are important.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

A schematic of the imaging technique from data acquisition to volumetric rendering. The steps include 1) acquiring respiratory- and ECG-gated 2D cine loops at 1000 FPS from a specific location, 2) translating the ultrasound probe using the attached motor and repeating step one at each location, 3) combining the extracted frames from each cine loop, dependent on their timing within the cardiac cycles, to create 3D volumes at each respective time-point, and 4) temporally concatenating said volumes to create 4D datasets

Fig. 2.

Three axis views of a healthy (top) and infarcted (bottom) mouse left ventricle taken at end-diastole. Ultrasound images of the infarcted LV were obtained four-weeks post-LAD coronary artery ligation. Papillary muscles are clearly seen in the short-axis view as extensions into the LV lumen (yellow arrows). Infarcted myocardial tissue is outlined in red. Green crosshairs highlight the center of the visualized structure. AO = aortic outflow. Scalebar = 1 mm

Fig. 3. 3D representations of the left ventricular lumen (red) and myocardial wall (blue) at end-diastole (left) and peak-systole (right) are shown for both healthy and infarcted mouse hearts. The asymmetrical anterior apical infarct is associated with wall thinning and dyskinesia of the necrotic myocardium at peak-systole. Scalebar = 1 mm

Fig. 4.

Box-and-whisker plots showing percent difference in a) end-diastolic volumes (EDV), b) peak-systolic volumes (PSV), c) stroke volumes (SV), d) ejection fraction (EF), and e) cardiac output (CO) measured from the specified techniques with respect to those measured from MRI. The upper and lower boundaries of the box plots correspond to the first and third quartile values of the dataset. Note that compared to conventional 2D techniques, cardiac parameters measured directly using the 4D technique aligned more closely to those measured from gold-standard MRI

Fig. 5.

Three-axis views of a representative mouse dissecting abdominal aortic aneurysm (AAA) at diastole. Black arrows point to the formation of intramural thrombus. Yellow arrow points to the true lumen, and dashed red lines outline the boundary of the AAA. Green crosshairs highlight the center of the visualized structure. Scalebar = 1 mm

Fig. 6.

a) 3D volume renderings of a representative mouse dissecting AAA during systole (blue) and diastole (red) are shown overlaid together, with the aneurysm positioned at the center. Enlarged subpanels demonstrate greater wall motion in the proximal and distal regions of the dissecting AAA, when compared to aneurysmal regions. b) Plots of Green-Lagrange maximum circumferential strain for the presented aorta, plotted relative to the abdominal aorta positions, highlight elastic walls located proximal and distal to the stiff aneurysm. c) Averaged Green-Lagrange maximum circumferential strain for dissecting AAAs ($n=7$) is plotted along normalized abdominal aorta positions, showing a stiffer aneurysmal region with reduced strain. Positions from the center of the aneurysms are normalized to half the aneurysm length so that the normalized position corresponding to the aneurysm falls within the -1 and 1 position. Data are shown as mean $\pm$ standard deviation. Scalebar = 1 mm

Table 1

Summary of Different Experimental Mouse Groups

Gender		Male	
Genotype		WT	apoE ^{-/-}
Study		Cardiac	Abdominal Aorta
Group	Healthy	Infarcted	Aneurysm
n	11	6	7
Age (weeks)	37 ± 20	15 ± 3	19 ± 2
Weight (g)	35 ± 8	29 ± 2	30 ± 1

Table 2

Summary of the Bland-Altman percent difference analysis of the 4D Ultrasound, Simpson, and Teicholz techniques with respect to MRI. EDV = end-diastolic volume, PSV = peak-systolic volume, SV = stroke volume, EF = ejection fraction, and CO = cardiac output

Parameters	Healthy			Infarcted		
	Statistics	Simpson	Teicholz	4D	Simpson	Teicholz
Cardiac						
EDV (μL)	Median [Q ₁ , Q ₃]	-16.2 [-21.8, -12.8]	-6.8 [-24.0, 7.5]	1.6 [0, 9.7]	-18.5 [-37.1, -8.3]	11.1 [-22.9, 26.3]
PSV (μL)	Median [Q ₁ , Q ₃]	-30.5 [-41.4, -16.5]	-22.1 [-26.2, -9.4]	1.5 [-7.2, 3.1]	12.2 [-16.1, 40.0]	1.5 [-35.3, 15.5]
SV (μL)	Median [Q ₁ , Q ₃]	-14.0 [-19.9, -7.8]	-2.6 [-10.6, 12.6]	5.1 [-11.8, 27.7]	-59.9 [-92.4, -34.9]	31.9 [-3.8, 58.3]
EF (%)	Median [Q ₁ , Q ₃]	5.3 [1.0, 8.9]	4.3 [1.2, 7.0]	2.3 [-10.9, 17.3]	-38.5 [-86.4, -6.6]	30.2 [-8.4, 50.6]
CO (mL/min)	Median [Q ₁ , Q ₃]	-14.0 [-19.9, -7.8]	-2.6 [-10.6, 12.6]	5.2 [-11.8, 27.7]	-51.9 [-82.8, -30.3]	38.6 [1.7, 76.4]