High Resolution X-ray Microscopy Using Digital Subtraction Angiography for Small Animal Functional Imaging

by

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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biomedical Engineering in the Graduate School of Duke University

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ABSTRACT
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Abstract

Research using mice and rats has gained interest because they are robust test beds for clinical drug development and are used to elucidate disease etiologies. Blood vessel visualization and blood flow measurements are important anatomic and physiologic indicators to drug/disease stimuli or genetic modification. Cardiopulmonary blood flow is an important indicator of heart and lung performance. Small animal functional imaging provides a way to measure physiologic changes minimally-invasively while the animal is alive, thereby allowing for multiple measurements in the same animal with little physiologic perturbation. Current methods of measuring cardiopulmonary blood flow suffer from some or all of these limitations—they produce relative measurements, are limited to global or whole animal or organ regions, do not provide vasculature visualization, limited to a few or singular samples per animal, are not able to measure acute changes, or are very invasive or requires animal sacrifice. The focus of this work was the development of a small animal x-ray imaging system capable of minimally invasive real-time, high resolution vascular visualization, and cardiopulmonary blood flow measurements in the live animal. The x-ray technique used was digital subtraction angiography (DSA). This technique is a particularly appealing approach because it is easy to use, can capture rapid physiological changes on a heart beat-to-beat basis, and provides anatomical and functional vasculature information. This DSA system is special because it was designed and implemented from the ground up to be optimized for small animal imaging and functional measurements. This system can perform: 1) minimally invasive in vivo blood flow measurements, 2) multiple measurements in the same animal in a rapid succession (every 30 seconds–a substantial
improvement over singular measurements that require minutes to acquire by the Fick method), 3) very high resolution (up to 46 micron) vascular visualization, 4) quantitative blood flow measurements in absolute metrics (mL/min instead of arbitrary units or velocity) and relative blood volume dynamics from discrete ROIs, and 5) relative mean transit time dynamics on a pixel-by-pixel basis (100 µm x 100 µm). The end results are 1) anatomical vessel time course images showing the contrast agent flowing through the vasculature, 2) blood flow information of the live rat cardio-pulmonary system in absolute units and relative blood volume information at discrete ROIs of enhanced blood vessels, and 3) colormaps of relative transit time dynamics. This small animal optimized imaging system can be a useful tool in future studies to measure drug or disease modulated blood flow dynamics in the small animal.
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List of Abbreviations

$\varphi(E)$  X-ray flux
CO           Cardiac output
CCD          Charged coupled device
DSA          Digital subtraction angiography
FPGA         Field programmable gate array
kVp          X-ray tube potential
mA           X-ray tube current
ms           X-ray exposure time
MOSFET       Metal oxide semiconductor field effect transistor
SdNR$^2$     Signal difference to noise ration squared
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1. Introduction

1.1 Small Animal Opportunities and Challenges

The use of small animals in research has grown in recent years because rodents are very well genetically characterized and serve as robust test beds for drug and disease models\(^1\). While current methods using ex-vivo or histology techniques can provide high spatial resolution, they require animal sacrifice and thus require large study numbers. In addition, the living physiology is no longer present in a fixed microtome slice. The use of MRI microscopy, microCT, microXray, microPET, optical imaging, and micro-ultrasound for small animal imaging has grown in interest because of the ability to capture data in the live animal minimally invasively, thus allowing for serial studies in the same animal (large populations are no longer required) and in-vivo anatomical/physiological measurements. Small animal imaging has unique requirements over clinical imaging in areas of spatial resolution and temporal sampling. The bodyweight of a typical rat is ~250g, 325X smaller than a human and the rat has a rapid ~450 beats per minute heart rate, 6.25X faster than a human. So the imaging system needs to have spatial resolutions in the micron range (pixel pitch ≤100 µm\(^2\), representing the rat distal pulmonary artery branchings\(^2\)) and sampling speeds in the millisecond range (≥7.5 frames per second, based on 450BPM heart rate) to be able to capture rapid physiological changes on a heart beat-to-beat basis. The physiological system of interest in this work was cardio-pulmonary blood flow because it is an important indicator of physiologic state/response to drug/disease stimulus. The focus of this work was the development of a small animal x-ray imaging system capable of minimally invasive real-time, high resolution vascular visualization, and cardio-pulmonary blood flow measurements in the live animal.
1.2 Previous Work and Limitations

Cardio-pulmonary blood flow is an important indicator of physiologic state/response to drug/disease stimulus. Current methods for measuring cardio-pulmonary blood flow include: Fick, thermodilution, magnetic flowmetry, microspheres, Doppler ultrasound, MRI, CT, and PET\textsuperscript{3-13}. The Fick method is the gold standard for measuring cardiac output in absolute units (mL/min). However, the Fick method is a global, whole body measurement (non-regional) and is limited to a small number of sample acquisitions since it requires blood withdrawals of 1-2mL for each measurement. Multiple measurements can be made in the same rodent using thermodilution, an advantage over the Fick method, but the reported values are relative measurements. Magnetic flowmetry can produce absolute units (when properly calibrated) but is very invasive (a midline sternotomy is commonly performed to gain access to the aorta for cardiac output measurements). In addition, internal vessels of the lung are out of reach for the probe placement because the pulmonary vessel branches are within the lung. The use of microsphere can produce regional blood flow information, a benefit over previous methods, but the results are relative values (commonly in concentration). In addition, only a few acquisitions can be performed in the same animal and this technique requires animal sacrifice. Doppler ultrasound, MRI, CT, and PET offer non-invasive solutions that allow for multiple measurements in the same animal and vascular visualization. Ultrasound offers high temporal sampling but is limited in measuring parenchymal blood flow because of the air in the lungs. In addition, the results are in terms of velocity (cm/min) rather then flow (mL/min). This characteristic makes the Doppler ultrasound measurements dependent on vessel size. CT and MRI are both used clinically. CT perfusion is a very active area of research\textsuperscript{14-16}, but scaling CT to the resolution necessary for small animals requires much longer scan times (10’s of seconds to minutes) which is
too long to capture rapid, acute changes in blood flow. Several different methods have been developed for perfusion MRI based on flow or the use of injectable contrast agents \(^{11, 17, 18}\). But these methods are also challenging when translating to the spatial and temporal resolution required for small animals and calibration can be particularly problematic.

This work describes a small animal x-ray digital subtraction angiography (DSA) system as a solution to the shortcomings experienced in other imaging techniques. DSA is a particularly appealing approach because it is easy to use, and it can capture rapid physiological changes on a heart beat-to-beat basis \(^{19}\). In DSA, a sequence of x-ray images is acquired pre- and post-vascular contrast injection. The pre-contrast injection images are averaged to create a mask from which the post-contrast injection images are subtracted, resulting in enhancement and visualization of the blood vessels, while suppressing the background anatomy. In previous small animal and canine x-ray angiography work \(^{20-25}\), the x-ray technologies used included film and digital detectors with a range of spatial resolutions and imaging speeds. A comparison of the x-ray technology is in Table 1.
Table 1: Comparison of previous work with this work in terms of spatial and temporal resolution (frame per second, fps).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Modality, Detector</th>
<th>Spatial Resolution</th>
<th>Temporal Resolution</th>
<th>Imaging Limitations/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longo et al.\textsuperscript{22}</td>
<td>X-ray film</td>
<td>1 sample</td>
<td>1 sample only</td>
<td></td>
</tr>
<tr>
<td>Rockman et al.\textsuperscript{25}</td>
<td>X-ray fluoroscopy</td>
<td>223 x 223 $\mu$m estimated</td>
<td>30 fps</td>
<td></td>
</tr>
<tr>
<td>Bhargava et al.\textsuperscript{20}</td>
<td>X-ray fluoroscopy</td>
<td>&gt; 60 x 60 $\mu$m estimated</td>
<td>60 fps</td>
<td>A/D conversion post image acquisition, low spatial resolution</td>
</tr>
<tr>
<td>Ono et al.\textsuperscript{23}</td>
<td>X-ray fluoroscopy</td>
<td>223 x 223 $\mu$m at best</td>
<td>30 fps</td>
<td></td>
</tr>
<tr>
<td>Hamamatsu Photonics K.K.\textsuperscript{26}</td>
<td>X-ray image intensifier</td>
<td>120 x 120 $\mu$m at best</td>
<td>30 fps</td>
<td></td>
</tr>
<tr>
<td>GE Healthcare\textsuperscript{27}</td>
<td>Flat panel x-ray digital detector</td>
<td>625 x 625 $\mu$m at projection</td>
<td>2.5 fps</td>
<td>Low temporal and spatial resolutions</td>
</tr>
<tr>
<td>Philips et al.\textsuperscript{28}</td>
<td>X-ray CCD</td>
<td>12 x 12 $\mu$m</td>
<td>0.14 fps</td>
<td>Low temporal resolution</td>
</tr>
<tr>
<td>Kobayashi et al.\textsuperscript{21}</td>
<td>Synchrotron</td>
<td>7 x 7 $\mu$m</td>
<td>30 fps</td>
<td>Required synchrotron facility</td>
</tr>
<tr>
<td>This Work, ImageStar (Photonic Science, East Sussex, England)</td>
<td>X-ray CCD</td>
<td>46 x 46 $\mu$m</td>
<td>10 fps</td>
<td>Fulfills temporal requirements at high spatial resolution</td>
</tr>
</tbody>
</table>

Longo et al.\textsuperscript{22} used a clinical mammography film system and thus were only able to achieve single samples. Work by Rockman and Ono\textsuperscript{23,25} obtained much higher sampling rates, up to 60 frames per second, but was lacking in spatial resolution and required conversion from analog video tape to a digital medium for subtraction and processing. The A/D conversion can introduce additional system noise and lead to image...
degradation. A sample of Ono’s work in 1994 compared to this work is shown below (Figure 1):

Figure 1: DSA images of the rat cardio-pulmonary system from Ono et al. and this work. Note the dramatic improvement in spatial and contrast resolutions that allow for visualization of the pulmonary artery branchings.

The estimated resolution in Bhargava’s work was >60µm² and the sampling rate was 60 frames per second. This was quite an accomplishment at the time. An advantage of the detector used in this study was the bit-depth, 14-bits, which was 64X more than the 8-bits in Bhargava’s work. The most current Hamamatsu Photonics image intensifier could produce higher resolutions but it is still ~9X less than the detector used for this work and requires analog to digital conversion. The GE Healthcare HiLight Matrix III, a current flat panel detector for many GE clinical CT systems, provides fully digital image information. This is an advantage over the previous technologies, but the spatial resolution is ~185X less than the detector used for this work. The reconstructed CT images can have a higher spatial resolution, up to 350 µm³, but
operates at 2.5 frames per second—too slow to capture the rapid physiological changes in a beating rodent heart (~600 beats per minute in the mouse and ~450 beats per minute in the rat). Using an x-ray CCD detector, Philips et al. 28 were able to achieve very high spatial resolution, 12 x 12 µm, using an indirect digital detector but required ~7 seconds to capture a full field of view image. Very high resolutions (7 x 7 µm) and temporal sampling (30 frames per second) was achieved by Kobayashi et al. 21 but required a synchrotron facility and thus routine studies were not possible. This work used a CCD detector with a 46 x 46 µm pitch and a 15 mg/cm² (~45 µm-thick) Gd₂O₂S scintillator and could provide acquisitions up to 10 frames per second. The spatial and temporal resolution specifications were at a level fitting for live rodent imaging on a heartbeat-to-heartbeat basis while providing high spatial resolution information.

The most important element of the work reported in this dissertation is the total system optimization: This system was optimized in terms of x-ray physics, physiological support and measurement, and system integration for the small animal. In previous work, 20-25, 29-31 this was not the case. Examples include: 1) the radiographic spectra was not optimized to produce the best contrast and SdNR², 2) the images were acquired asynchronous with cardiac or ventilatory cycles, which resulted in limited precision in measuring physiologic changes and significant subtraction artifacts, and 3) the contrast injections were manually given at low or varying flow rates with injection volumes up to 50% of the total blood volume (this would alter the physiology unfavorably). These deficiencies in prior works made quantitative measurements of blood flow unreliable.
1.3 Small Animal X-ray DSA Imaging Solution for In-Vivo Quantitative Blood Flow Measurements

This work describes a small animal x-ray digital subtraction angiography (DSA) system as a solution to the shortcomings experienced in other imaging techniques. DSA is a particularly appealing approach because it is easy to use, and it can capture rapid physiological changes on a heart beat-to-beat basis. In DSA, a sequence of x-ray images is acquired pre- and post-vascular contrast injection. The pre-contrast injection images are averaged to create a mask from which the post-contrast injection images are subtracted, resulting in enhancement and visualization of the blood vessels, while suppressing the background anatomy. This inherently gives DSA a high contrast to noise ratio and combined with the physiological driven image acquisition (3.5.1 Biological Pulse Sequence), eliminates subtraction and motion artifacts. From the DSA images, quantitative blood flow and relative blood volume information can be obtained over specific vessels, and relative mean transit time information on a pixel-by-pixel basis. This system is unique because of the combinations of high spatial and temporal resolution imaging, optimized x-ray spectra, high-precision x-ray contrast agent injection, physiological support, and quantitative blood flow analysis. The latter four aspects are described in further detail in Chapter 3 because they were designed and built in-house. The selection of a high spatial and temporal resolution detector is described below. The primary detector specifications important to this optimized system were: spatial resolution, temporal sampling speed, electron well depth, dynamic range, and field of view (Table 2).
Table 2: X-ray digital detectors evaluated with particular attention to spatial resolution, sampling rate, electron well depth, digitization, and field of view (FOV).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Detector / Scintillator (thickness)</th>
<th>Spatial Resolution</th>
<th>Temporal Resolution</th>
<th>Electron Well Depth</th>
<th>Digitization</th>
<th>FOV</th>
<th>Imaging Limitations / Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>VelociCam VC200A$^{32}$</td>
<td>CCD</td>
<td>12 x 12 $\mu$m</td>
<td>20 fps</td>
<td>50,000</td>
<td>14-bit</td>
<td>-</td>
<td>No scintillator, low well depth</td>
</tr>
<tr>
<td>SITe S100A$^{33}$</td>
<td>CCD</td>
<td>12 x 12 $\mu$m</td>
<td>-</td>
<td>110,000</td>
<td>13-bit</td>
<td>-</td>
<td>No scintillator, low well depth</td>
</tr>
<tr>
<td>Cantega 2k$^{34}$</td>
<td>CCD</td>
<td>13.5 x 13.5 $\mu$m</td>
<td>5 fps</td>
<td>-</td>
<td>16-bit</td>
<td>2.76 x 2.76 cm</td>
<td>Frame rate too slow, small FOV</td>
</tr>
<tr>
<td>Keenview$^{35}$</td>
<td>CCD</td>
<td>23.5 x 23.5 $\mu$m</td>
<td>10 fps</td>
<td>-</td>
<td>12-bit</td>
<td>3.23 x 2.43 cm</td>
<td>Low bit-depth, small FOV</td>
</tr>
<tr>
<td>Philips et al.$^{28}$</td>
<td>CCD/GdOx (45$\mu$m)</td>
<td>12 x 12 $\mu$m</td>
<td>0.14 fps</td>
<td>600,000</td>
<td>16-bit</td>
<td>4.9 x 8.6 cm</td>
<td>Frame rate too slow</td>
</tr>
</tbody>
</table>

This Work, ImageStar (Photonic Science, East Sussex, England) | CCD/GdOx (45$\mu$m) | 46 x 46 $\mu$m | 10 fps | 250,000 | 14-bit | 9.5 x 9.5 cm | Best combinations of spatial/temporal resolutions, electron well depth, and FOV |
There were many vendors\textsuperscript{28, 32-35} that offered high spatial resolution detectors, all with pixel pitch <50\(\mu\text{m}\)^2. However, only some (VelociCam and Keenview)\textsuperscript{32, 35} could provide frame rates that were \(\geq 7.5\) per second (rat heart rate\(\sim 450\text{BPM}\)). VelociCam had a very shallow electron well depth and thus could only support low x-ray flux applications. However, rapid heartbeat-to-heartbeat imaging calls for high flux x-ray exposures\textsuperscript{36}. The SITe S100A had \(\sim 2X\) the well depth as the VelociCam but both of these cameras did not provide a scintillator that would convert x-ray photons into light photons for capture and digitization. Although custom scintillators could be retrofitted onto these cameras, there would be significant image noise from scintillation light scatter. The ImageStar camera (Photonic Science, East Sussex, England) used in this work mitigates the amount of light scatter because the scintillator is directly bonded to fiber optics that directly channel the scintillated light to the CCD. The Keenview camera had a low bit-depth (12-bits, compare to \(\geq 14\)-bits from the other vendors) and thus could only provide 4096 analog digital units (ADU) instead of \(\geq 16,384\) ADU, thus limiting the low image contrast detectability. In addition, the imaging field of view (FOV) could not accommodate an entire rat. The Cantega 2k had the same FOV limitation. The camera used by Philips et al., had very high spatial resolution and deep electron well depth. In addition, the FOV was much larger (42 cm\(^2\) vs. \(\sim 8\) cm\(^2\)) than the other vendors and could fit an entire rat. However, this camera required 7 seconds to capture a full image. The CCD detector (ImageStar, Photonic Science, East Sussex, England) used in this work had a 9.5 x 9.5 cm field of view with a 46 x 46\(\mu\text{m}\) detector pitch and a 250,000 electron well depth. The FOV was large enough for an entire rat and the pixel resolution fine enough for capturing distal branching of the rat pulmonary artery\textsuperscript{2}. The frame rate could provide heartbeat-to-heartbeat imaging at high levels of digitization (14-bit) and the large well depth could support the high flux x-ray exposures. The ImageStar provided
the best combinations of spatial/temporal resolutions, electron well depth, and FOV that make it suitable for small animal x-ray imaging.

This small animal optimized DSA system can perform: 1) minimally invasive in vivo blood flow measurements, 2) multiple measurements in the same animal in a rapid succession (every 30 seconds—a substantial improvement over singular measurements that require minutes to acquire by the Fick method), 3) very high resolution (up to 46 micron) vascular visualization, 4) quantitative blood flow measurements in absolute metrics (mL/min instead of arbitrary units or velocity) and relative blood volume dynamics from discrete ROIs, and 5) relative mean transit time dynamics on a pixel-by-pixel basis (100 µm x 100 µm). The end results are 1) anatomical vessel time course images showing the contrast agent flowing through the vasculature, 2) blood flow information of the live rat cardio-pulmonary system in absolute units and relative blood volume information at discrete ROIs of enhanced blood vessels, and 3) colormaps of relative transit time dynamics. What is lacking in the previous work constitutes the three pillars of this work—optimized small animal digital radiography, quantitative physiological function measurement, and system integration. In specific, the components involved in creating an optimal system designed explicitly for small animal DSA are: x-ray beam optimization, a high precision micro power contrast injector, small animal physiological support and monitoring, quantitative physiological measurements, digital subtraction techniques, an image archival system, and system integration enabling physiologic event driven imaging. The integration of all of these elements into an operational platform designed for small animals promises some exciting new imaging alternatives for functional vascular imaging not possible with existing small animal imaging systems.
2. X-ray Imaging Background

2.1 X-ray Production

X-ray production and interaction theory described below are from Johns and Cunningham\textsuperscript{37} and Curry\textsuperscript{38}. Figure 2 shows a schematic of an x-ray tube. Current flows through a filament in the cathode causing electrons to boil off. An electrical potential difference pulls the electrons to the anode at a very high speed. The interaction of the electron with the anode creates x-rays.

![Figure 2: X-ray tube schematic](image)

Characteristic and general radiation (Brehmsstrahlung) processes are electron to atom interactions where diagnostic x-rays are generated. In characteristic radiation, an accelerated electron strikes the inner shell electron of an atom with enough force, $>$ binding energy, to eject the electron of the atom. Both electrons leave the atom for further interaction with other atoms. A photon is released as electrons in higher orbital shells “fall in” to fill the void in the lower orbital shell. The released photon observes the characteristic radiation emission of the matter that is specific to the energy difference between the higher and lower orbit shells. For example (Figure 3), an 80 keV electron impinges on a tungsten K-shell electron. The incoming electron has more energy than the binding energy of the tungsten K-shell electron (70 keV) and so the K-shell electron is
ejected and leaves the atom with the impinging electron. For energy stability, an outer shell electron fills in the void. If the replacement electron is from the L-shell, a photon is emitted with the binding energy difference (59 keV) between the K-shell (70 keV) and L-shell (11 keV). This high-energy photon can then interact with other atoms.

Figure 3: Characteristic radiation production.

In Brehmsstrahlung (Figure 4), the electron undergoes a directional change when it passes the positive nucleus of an atom. The energy that the electron loses during the directional change is emitted as a high-energy photon. These photons will have a range
of energies depending on the electron directional change and is a source of the polychromatic x-ray output. This high-energy photon can then interact with other atoms.

Figure 4: Brehmsstrahlung

2.1.2 Photon to Atom interaction

Photons, generated by Brehmsstrahlung for example, can interact with matter in four ways: Rayleigh or coherent scattering, Compton effect, photoelectric effect, and pair production. From the four, the photoelectric and Compton effects are the dominant interactions for diagnostic x-rays. In the Compton effect (Figure 5), an incident photon strikes an outer shell electron. There is energy deposition such that the photon decreases its wavelength and changes direction while the electron is knocked out of its valence shell.
Figure 5: Compton scattering

The scattering angle and change in photon wavelength follows

\[ \lambda_1 - \lambda_0 = \frac{(1 - \cos \theta)h}{m_e c} \]

where \( \lambda_0 \) and \( \lambda_1 \) are the incident and resulting photon wavelengths, respectively
\( \theta \) is the scattering angle
\( h \) is Planck’s constant which is \( 6.62 \times 10^{-34} \text{ J} \cdot \text{s} \)
\( m_e c \) is the rest energy of the valence electron

**Equation 1: Compton effect**

The exiting photon and electron can then further interact with other matter. In the photoelectric effect (Figure 6), a photon knocks out an inner-bound electron and a photon observing the characteristic radiation of the material is emitted when electrons from other shells fill in the void.
A trend of the photoelectric effect is that the mass attenuation coefficient is directly proportional to the third power of the attenuating material’s atomic number while being inversely proportion to the third power of the photon energy. In addition, there are “absorption edges” that correspond to the binding energies of the electron. As the mass attenuation coefficient drops with an increase in photon energy, there are discrete increases in attenuation when M, L, and K shell electrons are expelled. This is similar to the characteristic radiation generated in electron-atom interactions described above. Ejected electrons from the photoelectric effect and Compton scattering can then interact with other matter to produce characteristic radiation and Brehmsstrahlung. The process of electrons and photons interacting with atoms repeats a number of times as the x-rays are generated and pass through filters, the specimen, contrast agent, and finally reach the detector. In the travel through materials, the x-rays are attenuated following Beer’s Law, described as
\[ I = I_0 e^{-\mu t} \]

where \( I \) and \( I_0 \) are the X-Ray fluxs before and after attenuation, respectively
\( \mu \) is the material specific attenuation coefficient
\( t \) is the attenuator thickness

**Equation 2: Beer’s Law**

At the energies used for small animal DSA (~80kVp), the dominant x-ray cross sections in absorption are Compton scattering (primarily for the soft tissue) and the photoelectric effect (primarily for the iodine contrast agent and the gadolinium scintillator). Figure 7 shows the absorption cross sections (Compton, photoelectric effect, and their totals) of soft tissue, iodine (contrast agent), and gadolinium (scintillator in detector). Note how the photoelectric effect dominates in the x-ray absorption cross section for iodine and gadolinium because of their high atomic number (\( Z_I = 53, Z_{Gd} = 64 \)) while Compton scattering only has some effect for the tissue (\( Z_{\text{Tissue}} \approx 7 \)). The x-ray cross sections and the materials involved (tissue, iodine, and gadolinium) form the basis for the optimization of the x-ray technique discussed in 3.1.1 X-ray Beam Optimization.
Figure 7
Figure 7: Photoelectric, Compton, and total mass attenuation coefficients for soft tissue, iodine, and gadolinium as a function of energy. The energy levels (keV) are what are used in this small animal DSA system. Note how the photoelectric effect dominates in the x-ray absorption cross section for iodine and gadolinium. The photoelectric effect curve is almost superimposed on the total attenuation curve. Because of the low atomic number ($Z \approx 7$) of soft tissue, only here does the Compton effect play some role.
2.2 X-ray Detection

A significant leap in x-ray detection from film to digital detectors has allowed for higher spatial and contrast resolution and the possibility for new imaging modalities like CT. The description below is for indirect digital detectors, a specific kind of digital detector used in this work. The indirect x-ray detector (Figure 8) converts photons to visible light through a phosphor/scintillator.

![Figure 8: Schematic of indirect detector](image)

A x-ray photon is converted to visible light by the scintillator. The light is then piped to a CCD for digitization.

Commonly, the scintillating material is CsI or a Gd phosphor and the digital sensor is CCD or CMOS based. The justification for the selection of a Gd based CCD detector in this work will be described below. The k-absorption (33 keV) of the CsI phosphor crystals better match the x-ray attenuation of the iodine contrast agent (33 keV) than the Gd-based phosphor (Figure 9). So pre- and post-contrast x-ray images will have a larger difference and thus higher image contrast.
Figure 9: Attenuation coefficients of iodine (contrast agent), CsI (detector) and Gd$_2$O$_2$S (detector). Note the nearly identical curve between the iodine and CsI.

In addition, the CsI crystal inherently forms light pipes that deliver the converted light photon to the digital detector. However, the fabrication of CsI is challenging because of crystal defects, and thus elevates the cost of CsI above Gd and limits vendors. As seen in the previous chapter, the combination of many detector specifications led to the current detector choice where these requirements fulfill the imaging task at hand $^{36}$. The Gd$_2$O$_2$S phosphor in this work is directly bonded to fiber optics, which are then bonded to the CCD. So the visible light from scintillation gets piped directly to the digital sensor and allows for a tapering to produce an anti-blooming image and minimizes light scatter.

While CMOS detectors have higher read out rates than CCDs because many functions of electron to voltage conversion is performed on-chip, the increased amount of on-chip circuitry for each pixel increases the pitch between pixels (decreases spatial resolution) and limits the total amount of detection area (a smaller electron well depth). The CCD sensor in this work used a dual stage Peltier and water cooling system to reduce the electronic noise to be $< 0.5$ electrons/pixel/second. Other state-of-the-art CCD sensors that were considered had up to 7 electrons/pixel/second electronic noise $^{32}$. See chapter 1.3 for further details on the selection criteria (spatial resolution, temporal sampling
speed, electron well depth, dynamic range, and field of view) that led to the choice of the ImageStar camera to be used for this work.

An additional characteristic directly affecting x-ray detection is the Poisson statistical nature of electromagnetic radiation. Expressed mathematically in Equation 3 and below, the signal generated by an ideal detector that acts as an energy integrator is related to the detector area and the number of generated photons in a unit of time, $\varphi$.

Noise, as seen in the below equation, is modeled in a very similar fashion.

$$\text{Signal} = \int_{0}^{k_{vp}} \varphi(E)E \, dE$$
$$\text{Noise} = \sqrt{\int_{0}^{k_{vp}} \varphi(E)E^2 \, dE}$$

Equation 3: Signal and noise relation to x-ray flux

What is significant is that the noise is reduced by the square root of the signal. Thus, greater x-ray fluxes will produce better image quality. In addition, the noise is uncorrelated so averaging a set of pre-contrast images to create the mask from which post-contrast images are subtracted from will yield better noise statistics. Given the imaging task at hand and the x-ray physics background, the selection of the optimum x-ray technique in terms of the x-ray source, detector, and exposure characteristics (tube potential, current, and exposure time) was investigated and described in detail in 3.2.1 X-ray Beam Optimization.

3. X-ray System Overview

The radiographic system constructed for this work included a 0.3/1.0 mm focal spot tungsten (W) rotating anode tube (SRO 0950 ROT 350, Philips Medical Systems) and a 65 kW generator (EPS 65RF, EMD Technologies, Saint-Eustach, Quebec, Canada). The tube was mounted on a C arm. The tube provided short (10ms), high flux exposures.
A flexible carbon fiber bed for rodents rests above a 95x95mm cooled CCD detector (Microphotonics ImageStar) with a 46x46µm pitch and a 15mg/cm² (~0.0045cm thick) Gd₂O₂S scintillator. A small animal ventilator supporting constant pressure or volume provides anesthetic and breathing control. Biological and system signals are monitored and recorded in a LabVIEW (National Instruments, Austin, TX) application. Contrast agent is supplied via a computer-controlled micro-injector that permits ~10 ms temporal precision and delivers volumes down to 6 microliters. The entire system is controlled by three linked PCs running LabVIEW. Figure 10 shows our system during a live animal study.

Figure 10: micro X-ray system
Exposures, ventilation, injection, and image capture can be individually synchronized allowing a variety of “biological pulse sequences”\(^1\). Acquired images are processed in a MATLAB (The MathWorks, Natick, MA) graphical user interface. The subtracted images are then archived with scan specific information for storage, post processing, viewing, and Internet distribution. Details of each component are described in the sections below.

### 3.1 Digital Radiography for the Small Animal

This section describes the two key radiography aspects of this project. The first is the x-ray physics involved in creating the highest SdNR\(^2\) image\(^2\). The second component describes the power micro-contrast injector\(^4\). This injector can deliver repeatable x-ray contrast agent at high flow rates and low volumes necessary for quality digital subtraction angiography images and the functional measurements.

#### 3.1.1 X-ray Beam Optimization

Some prior work in x-ray imaging has been done in mice and rats\(^20-25\). However, these studies did not address the optimization of radiographic spectra to produce the best contrast and SdNR\(^2\), one of the many components in creating an optimal system designed explicitly for small animal DSA in live rodents. Several scaling differences between mice and humans suggest that a Mo anode might be preferable to the more traditional approach of a W anode used for DSA. The absorption coefficient of I at 17.5-19.7 keV (the K emission of Mo) is actually 1.7% higher than the absorption coefficient at the K edge (33.2 keV) and 16.6% higher at 35 keV (Figure 11). The absorption coefficient of Gd, the primary phosphor of the electronic detector used, is significantly higher at 17.5 keV than it is at the iodine K edge.
Figure 11: Attenuation coefficients for I (contrast agent) and Gd$_2$O$_2$S (detector phosphor) and detector response curve. Note that in region A, the attenuation coefficient of I is equal to or greater than that in regions B and C. Moreover, the detection efficiency for the Gd phosphor is substantially higher in A than in B and C.

Photon production efficiency (flux/mA) is substantially higher for (Mo) K emission than it is for W Bremsstrahlung at 17 keV.\textsuperscript{43} Finally, since the mouse and rat are quite thin, less than that of a human breast, one might hypothesize that penetration of the low energy K$_{\alpha}$ emission of Mo could provide a superior source for DSA imaging in the mouse.

Simulation and live rodent experimental verification were conducted. The three metrics used to determine the optimal x-ray source and technique were contrast, exposure-normalized SdNR$^2$, and tube-load limited SdNR$^2$, defined as
Contrast = \frac{S_{NC} - S_C}{S_{NC}}

\textbf{Equation 4: CNR}

and

\begin{equation}
\text{SdNR}_{(log)}^2 = \left( \log \frac{S_{NC}}{S_C} \right)^2 \frac{\sigma^2}{\left( \frac{1}{S_{NC}^2} + \frac{1}{S_C^2} \right)}
\end{equation}

\textbf{Equation 5: SdNR}^2

where \( S_C \) and \( S_{NC} \) are the detector signals with and without contrast agent, respectively. Derivation of the logarithmically subtracted SdNR\(^2\) can be seen in the Appendix. The exposure and tube-load limited SdNR\(^2\) were expressed in units of mm\(^2\)mR\(^{-1}\) (exposure limited, constant mR) and mm\(^2\)mAs\(^{-1}\) (tube-load limited, maximum mAs output provided by x-ray tubes at each kVp), respectively.

\textbf{3.1.1.1 Simulation}

Image contrast and SdNR\(^2\) were simulated using an established x-ray model.\(^{44, 45}\) All simulations were performed using the xSpect simulation program developed at Henry Ford Health Systems (Detroit, MI). The program uses semi-empirical models\(^{46-51}\) for generating x-ray spectra. Attenuation from iodinated contrast agent, soft-tissue, and inherent tube filtration was calculated following Beer’s Law.\(^{37, 52}\) Finally, the resulting spectra was integrated over the energy response function for the Gd phosphor of the phosphor-bound CCD detector employed in the experiments assuming an ideal detector that behaves as a perfect integrator of the energy spectrum.

A schematic of the simulation can be seen in Figure 12. The materials used in the simulation included a 0.045 mm-thick Gd\(_2\)O\(_2\)S scintillator for the detector; 13 mm- and
30 mm-thick soft-tissue absorbers, simulating the imaging situations for the mouse and rat, respectively; and a 0.28 mm diameter tube with I contrast agent of 37% concentration to represent the major vessels of the mouse and rat.²,₅³

Figure 12: The schematic shows the X-ray beam path used for the simulations. $S_C$ and $S_{NC}$ are detector signal outputs with and without contrast agent respectively.

To better reflect the experimental conditions, the simulations included additional Al filtration (2.8 mm and 0.6 mm for W and Mo, respectively) and a constant scaling factor to match exposure and attenuation measurements from our W and Mo tubes.

Simulations were performed over a range of tube potentials (18-70 kVp) for x-ray tubes with W and Mo targets. Since the tubes had different focal spot dimensions (0.6 and 1.0 mm for Mo and W, respectively), the data were normalized for source-detector distances that would match the geometric blur of the two tubes.

For clinical imaging, minimization of dose is a major concern. Thus, normalization with respect to exposure is an obvious metric for comparison. For rodent imaging, the higher respiratory and cardiac rates require the exposures to be much shorter than those for humans. At the same time, the higher spatial resolution and statistical considerations require higher exposures to maintain the SNR and CNR. The combination of these two effects suggests an alternative method for normalization.
against the exposure rate, i.e. normalization with respect to the available flux for a given time and tube loading (Figure 13). The data analysis included both approaches.

![Graph showing maximum current at 10ms vs KVP for W and Mo sources.](image)

**Figure 13**: The maximum current permitted for each tube for a standardized (10 ms) exposure is shown for each tube. Note the much higher currents possible for the W anode.

The simulation results show that contrast decreases rapidly with increase in tube potential up to the K-edge of I at 33 keV, where the contrast rises abruptly before falling off again at higher kVps (Figure 14 a and b). A rank inversion occurs soon after the K-edge of the Gd phosphor for mouse and rat. Below the K-edge of iodine, the source (W or Mo) has a significant impact on contrast. Above the K-edge, the impact of the anode material is less pronounced.
Figure 14
Figure 14: Contrast vs. kVp for Mo (a) and W (b) sources show a much higher contrast for lower kVp as one might expect with the highest contrast for Mo anode in the mouse (arrow). SdNR²/(mm² mR)-normalized to exposure vs. kVp for Mo (c) and W (d) sources shows two maxima for the thinner mouse. Lettered regions (a-d) within figures correspond to the live rat images shown in Figure 15. SdNR²/(mm² mAs) vs. kVp for Mo (e) and W (f) sources shows the higher values for the Mo anode for both the mouse and rat at all operating voltages indicating more efficient flux production of the Mo anode. SdNR²/mm², at max mA, for a 10ms exposure vs. kVp for Mo (g) and W (h) sources demonstrates superior performance of the W anode. The W source still produces a higher SdNR² than the Mo source, but only at energies above 60 kVp. Lettered regions correspond to live rat images shown in Figure 17.
At constant exposure, the SdNR² peaks at 20 kVp and 49 kVp for the mouse with both W and Mo sources, Figure 14 (c and d). The effect of the contrast agent increases the SdNR² at the K-edge of iodine as it does for contrast, Figure 14(a and b). At the optimum 49 kVp, the SdNR² is 23.6% (W) and 39.7% (Mo) higher for the rat and mouse imaging situations. At 49 kVp, the SdNR² is 78.8% (W) and 102% (Mo) greater for the mouse than the rat, and the peak at lower kVp disappears with thicker absorbers (rat). Figure 14 (e and f) show the SdNR² normalized against current. With increased tube potential, this metric also increases because the signal, following Poisson statistics, becomes significantly greater than that of noise. Virtually no difference exists between the two anodes for the rat at all kVp. Above 50 kVp, the SdNR² increases more rapidly for the mouse, reflecting the more efficient K production of the Mo anode and its ready penetration of the thinner animal. Following the initial hypothesis, on a per-mAs-basis, the Mo anode is superior to the W anode at all energies for small animal imaging. Note that the rate of increase (slope) is greater for the Mo anode than for the W anode for both mouse and rat. Figure 14 (g and h) were constructed using the tube loading charts (Figure 13) available for both of the specific tubes used in this study. Thus, Figure 14 (g and h) shows the SdNR² at maximum mA for a 10 ms exposure for geometries with equivalent spatial resolution.

The tube-loading chart (Figure 13) shows an inverted parabola with maximum mAs at 70 kVp. The point-by-point multiplication of the tube-loading chart with Figure 14 (e and f), results in a steady rise in current limited SdNR² (Figure 14 g and h) with a plateau at the higher kVps. The rise is more prominent and a plateau at a higher kVp is seen with the W compared to Mo tube. A major contributor to the shape of Figure 14(g and h) is the tube loading. As the space charge is overcome, the maximum tube current rises rapidly and then drops off as the thermal limitations of the tube come into play.
There is a dramatic rise in SdNR2 until 80 kVp for rodents, where SdNR2 plateaus for the W anode. The increase in SdNR2 for the Mo tube is more gradual and plateaus at tube potentials above 70 kVp for rodents. The W source is superior at all energies above 60 kVp.

3.1.1.2 Live Animal Experiment

To provide direct measure of image quality differences in a live rat DSA, images were acquired at 45 kVp and 70 kVp for both anodes under two separate conditions: 1) with the same entrance exposure and 2) with maximum tube loading. In the case of the same entrance exposure imaging, the raw images from the Mo tube were scaled to match the exposure of the W tube because the Mo tube generator had a coarse adjustment in mAs, by 25% steps. The relative SdNR$^2$ in the live rodent DSA images are reported of the left pulmonary artery at the same time point in contrast passage. The values were derived experimentally using Equation 5 where the noise is measured from an ROI in the corner of the image in which there is no structured background. Strictly speaking, this is not the true SdNR$^2$ since the photon flux will be higher in this region than in the region of the anatomical landmark (e.g. pulmonary artery). This method limits any variation between images arising from structure in the object.

Separate animals were used for the images acquired at the same entrance exposure and with maximum tube loading. Radiographic exposures, readout of the camera, and contrast agent injections (Isovue 370, 370mg iodine/mL, Bracco Diagnostics, Princeton, NJ) were synchronized at the same point in the cardiac cycle with end-respiration apnea. Because the heart rate of a rat is a rapid 450 beats per minute, 7.5 times that of a human, the exposure time was kept at ~10 ms to limit motion blur. A micro-injector, described in the next section, allowed the control of contrast injection with temporal precision of ~10 ms and volumes down to 6 ±1 microliters.
Pulmonary flow could be seen with contrast volumes as low as 1% of the total blood volume in the mouse and at less than 1% of the volume in the rat. Injections were spaced 10 minutes apart to allow clearance of the contrast agent, which limited complications from background concentrations of contrast in the blood. Separate experiments have been performed with the micro-injector demonstrating reproducibility of the time density curves for a given injection with minimal variations (correlation coefficient ≥ 0.9). Images were logarithmically subtracted and compared.

Figure 15 shows logarithmically subtracted DSA images of the rat thorax, taken at the same exposure at 45 kVp and 70 kVp during the same point in the cardiac and respiratory cycle following an injection of 50 microliters of Isovue 370 in the right jugular vein. Images were windowed to present comparable displayed contrast.
Figure 15: DSA images of the thorax of a live rat acquired at 45 kVP (a, c) and 70 kVP (b, d) at the same exposure using Mo (a, b) and W (c, d) anodes. While the Mo anode allows better visualization of distal pulmonary artery branchings, the W anode demonstrates improved visibility of the right ventricle (solid arrow) and proximal pulmonary artery branchings (dashed arrows). SdNR$^2 = 49$ (measured at the left pulmonary artery) for the W source at the low and high tube potentials. SdNR$^2$ values of 41 and 50 were measured for the Mo source operated at 45 kVP and 70 kVP, respectively.
Note the improved visibility of the proximal branching pulmonary arteries in the lungs (dashed arrows) and the increased opacification of the right ventricle (solid arrows) in Figure 15 with the W tube (c, d) relative to the Mo tube (a, b). The images taken using the same x-ray source at both tube potentials show similar image quality (a, b and c, d). The SdNR\(^2\) = 49 (measured at the left pulmonary artery) for the W source at the low and high tube potentials. SdNR\(^2\) values of 41 and 50 were measured for the Mo source operated at 45 kVp and 70 kVp, respectively. The effects of varied tube potential and anode material on contrast are consistent with our initial hypothesis and the body of work in mammography. If one corrects for scatter, the thickness of tissue does not have any effect on contrast. Only the thickness of the embedded I contrast agent has an effect on contrast apart from the tissue and contrast agent attenuation:

\[
\text{Contrast} = \frac{I_{NC} - I_C}{I_{NC}} = 1 - \frac{I_C}{I_{NC}} = 1 - \frac{I_o e^{-\mu_T(T-C)} - I_o e^{-\mu_C C}}{I_o e^{-\mu_T T}}
\]

\[
= 1 - e^{(\mu_T - \mu_C)C}
\]

\[\therefore \text{Contrast} \neq T\]

**Equation 6:** Image contrast is unrelated to the tissue thickness. It is only related to the contrast agent thickness.

Here, \(I_o\), \(I_{NC}\), and \(I_C\) are entrance, tissue attenuated, and tissue-contrast attenuated x-ray beams, respectively. \(T\) and \(C\) are the thicknesses, and \(\mu_T\) and \(\mu_C\) are the attenuation coefficients of the tissue and embedded contrast. The contrast is higher with the lower energy spectrum of the Mo than the W anode (Figure 14a and b). The higher attenuation coefficient of I at lower kVp gives rise to a marked increase in contrast below 26 kVp for both anodes. The K-edge of I contrast agent at 33 keV, causes a steep rise in contrast. As the tube potential is increased, the effective energy of both the Mo and W spectra continue to shift to higher energies above the iodine K-edge, causing the
contrast to drop. The results for SdNR$^2/(\text{mm}^2 \text{ mR})$ vs. kVp are shown in Figure 14 (c and d). As the tissue thickness increases from 1.3 cm (mouse) to 3.0 cm (rat), the peak in the SdNR$^2$ at 20 kVp disappears. The lower energy radiation simply does not adequately penetrate for rat studies. Yet, there remains a clear optimum kVp for both the mouse and rat at ~ 50 kVp. The SdNR$^2$ at constant entrance exposure is 23.6% (rat) and 39.7% (mouse) greater for the W anode than Mo anode at this optimal kVp. Images acquired at constant entrance exposure (~20mR) in Figure 15 agree well with the simulations; the higher contrast Mo source (a, b) allows better visualization of the distal pulmonary arteries while the proximal branchings of the pulmonary artery and the right ventricle are more apparent in the images acquired with the W (c, d) tube. The pulmonary artery shows more homogeneous enhancement using the W tube (Figure 15 dashed arrow). Subtle contrast differences seen in the Mo anode (dashed arrow in Figure 15a) are not as apparent with the W anode (dashed arrow in Figure 15c). Image improvements within the same x-ray source (a, b and c, d) are not significant. These effects are consistent with the simulation results in Figure 14 (c and d) and at most a ~20% difference in relative SdNR$^2$. Figure 14 (e and f) tell a somewhat different story when one takes into account the efficiency of x-ray production. As the accelerating potential increases, the efficiency of photon production increases more rapidly than the contrast decreases resulting in a net increase in SdNR$^2$ as would be expected. With this normalization, the Mo anode shows a slightly better performance than the W anode, consistent with our initial hypothesis. But the magnitude of this effect is small. And for the thicker (rat) specimen, there is virtually no benefit for the Mo anode.

Figure 17 shows logarithmically subtracted images of the rat lung, taken with 10 ms exposure at the maximum mA permissible at 45 kVp (a and c in Figure 17) and 70 kVp (b and d in Figure 17) at the same point in the cardiac and respiratory cycle with a
50-microliter injection of Isovue 370 into the right jugular vein. Images were windowed to present comparable displayed contrast as in reference.\textsuperscript{55}
Figure 16: DSA images of the thorax of a live rat acquired with 10 ms exposure at the maximum mA permissible at 45 kVp (a, c) and 70 kVp (b, d) using Mo (a, b) and W (c, d). The images demonstrate superior image quality (SdNR²) for the W anode operated at the higher potential. This is seen in the improved visibility of the right ventricular filling (solid arrows) and the ability to distinguish between the lung parenchyma and the pulmonary vessels (dashed arrows). W (SdNR² = 144) and Mo (SdNR² = 81) x-ray sources at high tube potential (70 kVp) have higher signal to noise ratio than at lower tube potential (45 kVp) for both the W (SdNR² = 36) and Mo (SdNR² = 49) x-ray sources. There was a 78% (70 kVp) and 294% (45 kVp) improvement in relative SdNR² for the Mo source, and a 400% improvement for the W source operated at 45 kVp.
The comparison of the relative noise levels and detail in the images confirm simulation results: the Mo x-ray source at the low 45 kVp produces better image quality (qualitatively and by SdNR$^2$ described below) as seen in Figure 17 (a) than the W source (c), while the W x-ray source produces better image quality (d) than the Mo source (b) at a higher (70 kVp) tube potential. In addition, comparisons of images taken at 45 kVp and 70 kVp with the same x-ray source (Figure 17 a, b and c, d) show operation at higher kVp to be better in both cases. While flux production is more effective for a given power for the Mo K production, the higher heat capacity of the W anode allows operation at significantly higher flux for the short exposures required to limit motion blur. The higher available flux from the W anode at 70 kVp has dramatic impact on our ability to detect the smaller vessels. Quantitative results agree well with the simulations: the W (SdNR$^2$ = 144) and Mo (SdNR$^2$ = 81) x-ray sources at high tube potential (70 kVp) have higher signal to noise ratio than at lower tube potential (45 kVp) for both the W (SdNR$^2$ = 36) and Mo (SdNR$^2$ = 49) x-ray sources. There was a 78% (70 kVp) and 294% (45 kVp) improvement in relative SdNR$^2$ for the Mo source, and a 400% improvement for the W source operated at 45 kVp. Note in particular, the improved visibility of the right ventricular filling (solid arrows) and the distinction between the pulmonary vessels and the lung parenchyma (dashed arrows) in Figure 17.

This work suggests the use of high flux x-ray exposures for high SNR. To ensure that the amount of radiation was acceptable for the small animal, dose measurements were made for the maximum tube loading case using MOSFET detectors (mobileMOSFET, LACO, Chesterland, OH). The dosimeter was calibrated using an ionization chamber (Electrometer Model 9015, Probe 10x5-6 was used for the W source, MDH Radcal, Monrovia, CA) with probes sensitive for even at the low Mo energies (Probe 10x5-6-6M-3). The figures reported represent the total integrated dose for a standard DSA study consisting of 30 frames of 10 millisecond exposures. The surface
dose was 3.17 cGy (Mo) and 0.99 cGy (W) at 45 kVp, and 4.85 cGy (Mo) and 5.32 cGy (W) at 70 kVp. While the dose is relatively high (5.32 cGy) at the surface, it is significantly less than what a typical commercial microCT scanner would impart and at most $1/100$ of the LD50/30 of a mouse, thus allowing sequential studies with confidence of little x-ray-induced damage to the small animal. Further detail of MOSFET based dosimetry is described in 3.4 Small Animal Dosimetry.

### 3.1.1.3 Summary

An experiment was conducted to find the optimal x-ray source—molybdenum or tungsten—and kVp to produce the highest quality small animal functional subtraction angiograms in terms of contrast-to-noise ratio (CNR) and signal-difference-to-noise ratio squared (SdNR²). Two limiting conditions were considered—normalization with respect to dose and normalization against tube loading. Image contrast and SdNR² were simulated using an established x-ray model. DSA images of live rats were taken at two representative tube potentials for the W and Mo sources.

Results show that for small animal DSA, the Mo source provides better contrast. However, with digital detectors, SdNR² is the more appropriate image quality metric. The W source operated at kVps $> 60$ achieved a higher SdNR² (Figure 14). The live animal experiment also confirmed this conclusion as seen in Figure 17. The highest SdNR² was obtained at voltages above 90 kVp. However, operation at the higher potential results in significantly greater dose and tube load. A reasonable tradeoff can be achieved at tube potentials at the beginning of the performance plateau, around 70 kVp, where the relative gain in SdNR² is the greatest.
3.2 High Precision Micro-contrast Power Injector

A computer-controlled power injector was built that allowed quantitative blood flow measurements through functional DSA imaging of mice and rats with minimal physiological impact. More information can be found here 42. Below is a summary of the motivation, methods, and conclusion of the findings.

The drive for higher spatial and temporal resolution to capture rapid physiologic changes has led to the development of the micro power contrast injector described in this paper. Prior work in mice and rats 20-25, 29-31, 59 has not fully optimized the methods for small animals. For example, previous images were acquired asynchronous with cardiac or ventilatory cycles, resulting in limited precision in measuring physiologic changes and significant subtraction artifacts. Contrast injections were given manually at low or varying flow rates. These deficiencies in prior work made quantitative measurements of blood flow unreliable. In addition, some studies used significant contrast injection volumes—up to 50% of the total blood volume and would alter the physiology unfavorably.

A number of injection parameters are crucial to produce a quality subtraction angiogram. These include a tight (temporal) bolus with a small volume and high flow rate combined with appropriate catheter placement 60-63. Large contrast volumes with slow flow at peripheral catheter locations result in spread and dilution of the contrast bolus. The bolus dilution contributes to increase in blood volume from contrast because one must inject more contrast agent to achieve adequate enhancement. This can lead to retrograde flow and the inability to separate left/right heart and lung 23, 25, 64, 65. The computer-controlled system described here can inject small volumes of contrast agent at high flow rates with high reproducibility at very precise times in the physiological cycles.
The system (Figure 17-i) is designed to inject precisely controlled amounts of contrast (Isovue 370, 370 mg iodine/mL, Bracco Diagnostics, Princeton, NJ) at high rate and at specific points in the breathing and cardiac cycles. Timing is controlled through LabVIEW software (National Instruments, Austin, TX) (Figure 17-ii) that allows reproducible event-driven sequences that link image acquisition, contrast injection, and physiologic control and monitoring. When a trigger is received (Figure 17-i, a), a pressurized N2 supply (b) pushes contrast through a zero dead volume direct lift solenoid valve (d) (Cole Parmer, Vernon Hills, IL). The trigger is provided by the LabVIEW software and a TTL-generating PCI-6602 counter/timer board (National Instruments, Austin, TX). This specific solenoid was chosen because it had five important characteristics: 1) it is able to withstand high pressures (up to 100 PSI); 2) it is easy to clean; 3) it supports high flow rates; 4) it holds no contrast volume in the valve chamber, which eliminates dead volume; and 5) it has a rapid (milliseconds) response time. These solenoid characteristics allow repeatable delivery of small injection volumes at high flow rates. A custom–tapered catheter was constructed for the mouse. A commercial catheter was used for the rat. Isovue 370 has a viscosity 20.9-times that of water making injections at high flow rates in rodent-sized catheters a challenge. To address this challenge, catheters were chosen for maximum lumen size, while still being small enough for cannulization. Tapered catheters were developed to support the required higher flow rates. Catheter lengths were shortened as much as possible to reduce flow resistance, while still allowing adequate mobility for the surgical cannulization procedure. For the mouse studies, the custom–catheter (Figure 18) was made from polyethylene (PE) tubing of various interconnecting sizes (PE-190, 90, 50, 10). In the rat studies, a shortened (13.5 cm from catheter tip to the end of the stub adapter) polyurethane catheter was used (PU-50 Chronic-Cath, CC-3P, Access Technologies, Skokie, IL).
The contrast resides in a heated reservoir (Figure 17-i, c) between the solenoid and N2 supply. The temperature is maintained at 37°C to reduce the viscosity of Isovue 370 from 20.9 to 9.4 mPa·s. The heating also alleviates temperature shock when injecting into the small animal. All the parts of the micro-injector have quick-disconnect fittings to allow quick cleaning and maintenance. The combination of the specific components allowed the design and implement of a micro-contrast power injector that could deliver small bolus injection volumes at high flow rates with computer controlled timing.
Figure 17: Micro-injector components (i): (a) trigger input, (b) air supply, (c) temperature-controlled contrast reservoir, (d) zero-dead-volume direct lift solenoid, and (e) rotating Luer fitting for connection to custom injector catheters. Pane (ii) shows a schematic overview of the x-ray system. A LabVIEW-based system integration allowed for reproducible event-driven imaging sequences that linked image acquisition, contrast injection, and physiologic control and monitoring.
Figure 18: The custom catheter (not to scale) designed for the mouse was constructed from a series of polyethylene catheter segments glued together cascading from a large lumen (PE-190) for supporting a high injection flow rate to a small diameter lumen (PE-10) so that cannulization was possible.

Bench-top and live animal x-ray imaging experiments were conducted to measure the injection volumes under various scenarios, the reproducibility of the injections, the smallest amount of contrast agent detectible by our system, and the physiologically-mediated effects of contrast injection. The optimum injection volumes and flow rates were found that allowed for visualization of non-overlapping, contrast-enhanced blood flow in the live animal.

3.2.1 Bench-top Experiment

Bench-top tests were conducted to characterize the micro-injector injection volumes and flow rates with various combinations of catheter lengths and lumen sizes, driving pressures, and length of time the solenoid valve was opened. Injection volumes and flow rates were determined by a mass-difference analysis. For each combination, the mass of 50-60 micro-centrifuge tubes was measured before and after the addition of injected contrast. The mass difference was divided by the contrast density (1.41 g/cm³), resulting in the volume of injected contrast. The flow rate was computed by dividing the newly found injection volume with the duration of the solenoid activation window, mathematically expressed as:
Avg. inj. vol. (µL) = \frac{1}{n} \sum_{i=1}^{n} \left( \frac{\text{mass}_{\text{contrast}} - \text{mass}_{\text{no contrast}}}{\text{density}_{\text{contrast}}} \right) 

where \( n = 50 \) to 60

**Equation 7: Average injection volume**

Avg. inj. flow rate (µL/ms) = \frac{\text{Avg. inj. vol.}}{\text{solenoid activation window}}

**Equation 8: Average injection flow rate**

Linear regression was applied to injection volume and flow curves in respect to varying solenoid active window at a constant 80 PSI driving pressure and varying driving pressure at a constant 50 ms solenoid active window. Coefficient of determination \( (r^2) \) values were found for the linear fits.

The characteristics of the micro-injector found in the bench-top experiments are shown in Table 3. Figure 19 and Figure 20 graphically show injection volumes and flow rates as a function of the solenoid active window at constant driving pressure and the performance when changing the driving pressure while keeping a constant solenoid active window. Each point is the mean of 50-60 measurements. The \( r^2 \) was > 0.99 for the linear regressions of injection volume vs. solenoid active time windows at a constant driving pressure. The flow rate remained constant from 50 to 200ms. The \( r^2 \) for the linear regressions that mapped injection volume and flow rate vs. driving pressure at a constant injection duration was also > 0.99. Figure 20 demonstrates this linearity.
Table 3: Micro-injector injection volume and flow rates for rat and mouse catheter, as a function of injection time, and driving pressure. Figure 3 and Figure 4 graphically represent these data.

<table>
<thead>
<tr>
<th></th>
<th>Mouse DSA</th>
<th>Rat DSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID (mm)</td>
<td>0.28 at tip</td>
<td>0.6</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>63</td>
<td>130</td>
</tr>
<tr>
<td>Window (ms)</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Pressure (PSI)</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Volume (µL)</td>
<td>9.2±1.9</td>
<td>12.5±1.4</td>
</tr>
<tr>
<td>Rate (µL/ms)</td>
<td>0.18±0.038</td>
<td>0.12±0.014</td>
</tr>
</tbody>
</table>
The micro-injector has high precision in that it could deliver linearly, reproducible injection volumes and flow rates (Figure 19 and Figure 20). This is an important characteristic to prevent overdosing (too much injected contrast volume) and in establishing a linear relationship between x-ray enhancement and physiological changes.

Figure 19: Mouse and rat catheter injection volumes and flow rates for various solenoid active window durations at constant driving pressure (80 PSI). Each data point consisted of 50-60 measurements. The curves include ±1 standard deviation for each data point (only the rat injection volume and mouse injection flow rate had standard deviation bars that were visible. For the other cases, the absolute variances were too small and are not visible). There is a linear response of injection volumes and flow rates to the solenoid active windows as seen by the $r^2$ values (>0.99) in a linear fit.
Figure 20: Injection volumes and flow rates for 5, 10, 20 and 80 PSI driving pressures at constant 50ms solenoid active window are shown for the rat. Each data point consisted of 50-60 measurements. The curves include ±1 standard deviation for each data point. There is a linear response of injection volumes and flow rates to the driving pressure as seen by the $r^2$ values (>0.99) in a linear fit.

A key factor in achieving the linear response is the design of the custom catheters. The catheters were designed for maximum flow rate, while still being small enough for cannulization. This was achieved by using the largest lumen possible for the vessel being cannulated followed by successively larger segments approaching the injector. The overall catheter lengths were as short as possible to reduce flow resistance, while still being long enough for surgical handling. At constant driving pressure, a linear increase in injection volumes occurs for both catheters with increasing injection time (Figure 19). The flow rate at constant driving pressure is independent of the solenoid active window. Figure 20 shows linear increases in injection volume and flow rate with increasing driving pressure at a constant injection time. Here, only the rat catheter was characterized because lowering the driving pressure for the mouse catheter decreases the
injection volume to a point where the standard deviation is > 25% of the injection volume.

### 3.2.2 Live Animal Experiment

For the live animal studies, images were acquired at every heartbeat with endexpiration apnea, with one contrast injection for each sequence. For the rat studies, pixels were binned at 2x2 producing an effective resolution of 92 µm x 92 µm. No binning was used for the mouse images resulting in an effective resolution of 46 µm. Images were logarithmically subtracted.

Experiments were conducted on the live animal to find the appropriate injection volumes and to characterize the performance of the micro-injector. Regions of interest (ROIs) included the pulmonary artery, lung parenchyma, left ventricle, and aorta. The heart, coronary arteries, and kidney vasculature were also imaged to show other uses of the injector.

A series of injection volumes, ranging from 50 to 1000 µL, was performed on the rat to define the linear range of enhancement of the blood vessels. Performance of the system was evaluated according to the following characteristics: limited increase in total blood volume (<10%), non-enhancement of overlapping structures; no enhancement due to second pass of contrast; a contrast-to-noise-ratio (CNR) ≥ 5 (Rose criterion). The CNR was measured by:

$$\text{CNR} = \frac{S_{SC} - S_C}{\sigma_{SNC}}$$

**Equation 9: Contrast-to-noise ratio**

where $S_C$ was the DSA signal in an enhanced blood vessel, $S_{NC}$ was the signal of the background, and $\sigma_{SNC}$ was the standard deviation in a region of the image in which there
is no structured background. This standard deviation is in part due to the Poisson statistical nature of x-ray production. The aorta was chosen for CNR measurements because in an anterior-posterior projection, this vessel does not overlap any other vessels.

Figure 21 shows enhancement of the pulmonary circulation in the rat, especially in the parenchyma, with increasing volumes of contrast. Note that injection volumes larger than 150 µL created overlap of enhanced vessels that can inhibit accurate measures of circulation. As the injection duration becomes longer than a heart cycle, there is an increase in the duration of the bolus, which increases overlap of enhanced vessels.
Figure 21: DSA time sequence images for the rat after contrast injection using 50, 100, 150, 200, and 1000 µL injection volumes (columns a to e) at the same points in its physiologic cycles of 430, 100, and 1400 ms (rows 1-3) in the same animal show increasing opacification at all time points with larger injection volumes. There was more enhancement of the distal vessels and parenchyma with larger injection volumes. However, too large a volume created retrograde flow (solid arrow in e1) and significant overlapping of enhanced structures (dotted arrow in d-3, and also e-2 and e-3). In addition, there was significant increase in total blood volume. These factors suggested injections volumes between 50-150 µL (columns a to c). This also allowed the contrast to be injected within one heartbeat.
The CNR between the aorta of the rat and the background as a function of time is shown for various injection volumes in Figure 22. As expected, the CNR increases with larger contrast volume. The rise and fall in the individual CNR curves is observed as the contrast enters and passes the region of interest in the aorta. Injection volumes $\geq 100 \, \mu\text{L}$ meet the Rose criterion for detection at their peak, i.e. a contrast to noise ratio $\geq 5$.

![Image](image.png)

Figure 22: The contrast-to-noise-ratio (CNR) in the subtracted image of the rat aorta peaks above 5 for injection volumes greater than 100 $\mu\text{L}$. The aorta was chosen because there is no overlap of contrast-enhanced vessels in the projection image. The dotted horizontal line represents the Rose criterion. This suggested injection volumes $\geq 100 \, \mu\text{L}$ would be suitable in the rat.

In the rat, injection volumes between 50-150 $\mu\text{L}$ produced significant enhancement of the pulmonary vasculature with injection volumes and durations that were physiologically reasonable and had limited enhancement overlap (Figure 21). Larger injection volumes created significant overlap of enhanced structured and increased total blood volume (dotted arrow in d-3, and also e-2 and e-3). However, Figure 22 suggests injection volumes $\geq 100 \, \mu\text{L}$, where the CNR meets the Rose criterion.
Therefore, a 100-150 µL contrast injection is recommended for the rat. The total blood volume in a 200 g rat is ~12 mL. Thus, this injection volume is ≤ 1.25% of the total blood volume\textsuperscript{69-72}. For the mouse, the renal vasculature was visualized with a 20 µL injection. The blood volume of the mouse is ~2 mL, so the injection volume is ~1% of the total blood volume.

Once an injection volume that produced good enhancement of the blood vessels was found, the variability was studied in physiology arising from the injection parameters. Six DSA runs were performed with injection volumes increasing from 50 to 150 µL and then back down to 50 µL in 50 µL gradations. The contrast injection was performed at the QRS of the cardiac cycle, and images were acquired at every QRS at end-expiration apnea. To measure the repeatability and any variability in imaging physiology arising from the injection parameters, a nonparametric deconvolution technique using singular value decomposition (SVD) was used to find relative pulmonary blood volumes (PBV), pulmonary blood flows (PBF), and mean transit times (MTT) of the left pulmonary artery, right lung parenchyma, left ventricle, and aorta.
Figure 23: Minimum intensity projection of a typical rat DSA run that included 30 images with regions of interest used for the time-density curve measurements shown in Figure 8 and singular value decomposition (SVD) based calculation of pulmonary blood volume, blood flow, and mean transit time. The pulmonary artery root was used as the arterial input function for the SVD calculations.
The SVD technique used was based on work done by Ostergaard et al. \(^{73-76}\) and is described in further detail below: The concentration of tracer (contrast agent) in distal vessel of interest (\(C_{VOI}\)) is the convolution of the input function (\(C_{AIF}\)) and the transport function, \(h(t)\).

\[
C_{VOI}(t) = C_{AIF}(t) \otimes h(t) = \int_0^t C_{AIF}(\tau) h(t-\tau)d\tau
\]

**Equation 10:** \(C_{VOI}\) is the convolution of an input function (\(C_{AIF}\)) and the transport function, \(h(t)\).

\(h(t)\) is the probability density function of transit times representing the vascular structure and the blood flow (\(F_t\)). Expanded, \(h(t)\) is:

\[
C_{VOI}(t) = F_t \int_0^1 C_{AIF}(\tau) \left( 1 - \int_0^1 h(\tau)d\tau \right) d\tau = F_t \int_0^1 C_{AIF}(\tau) R(t-\tau)d\tau
\]

**Equation 11:** Transport and residual functions.

where \(R(t)\) is the residue function and is the fraction of contrast agent still present in the vasculature at time \(t\). From the DSA data, \(C_{VOI}\) and \(C_{AIF}\) are the time density curves at a distal segment of the vasculature and the input function (pulmonary artery root), respectively. The goal is to solve for \(F_t\) and \(R(t)\). There are two general approaches to solve for these two variables—model dependent and model independent approach. In the model dependent approach, \(R(t)\) is estimated as a decreasing exponential function from tissue models/simulations. This allows one to directly solve for \(F_t\). The benefits of the model dependent approach is it is straightforward in solving and is stable in terms of noise sensitivity because \(R(t)\) is user defined. The drawback is that one assumes \(R(t)\) to be a certain exponential function. However, this a priori information may not be
available and furthermore, this function varies widely between major vessels and branchings, and in vessels with high and low flow. In the model independent approach, deconvolution techniques are used to determine R(t) along with F_t. The techniques here differ only in how they modulate the effects of noise in the calculations. A straightforward way of deconvolution is by the inverse Fourier transform:

\[ C_{VOI}(t) = F_t \int_0^t C_{AIF}(\tau)R(t-\tau)d\tau \]

\[ \Im\{C_{VOI}(t)\} = F_t \times \Im\{C_{AIF}(t)\} \times \Im\{R(t)\} \]

\[ F_t \times R(t) = \Im^{-1}\left\{ \frac{\Im\{C_{VOI}(t)\}}{\Im\{C_{AIF}(t)\}} \right\} \]

**Equation 12: Model-independent inverse Fourier approach.**

where \( \Im\{ \} \) denotes the Fourier transform and \( \Im^{-1}\{ \} \) denotes the inverse Fourier transform. So in this approach, the flow and residue can be found by taking the inverse Fourier transform of the ratios of two transforms at every time point of the AIF and VOI. This approach is very straightforward to solve but is very noise sensitive such that small, finite changes in \( C_{VOI} \) result in large changes in \( R(t) \). One can apply Fourier domain filtering schemes (e.g. Weiner filter) before the inverse Fourier transform to mitigate the noise impact. The approach used in this work was a model independent algebraic reformulation of the convolution integrals. In discretized fashion,

\[ C_{VOI}(t) = \int_0^t C_{AIF}(\tau)R(t-\tau)d\tau \]

\[ = \Delta t \sum_{i=0}^{j-1} C_{AIF}(t_i)R(t_j-t_i) \]

**Equation 13: Discretization of \( C_{VOI} \).**

where \( \Delta t \) is the sampling interval (because \( F_t \) is a scalar, it is included as part of \( R(t) \) and is solved later). Furthermore, this discretization can be expressed in matrix form:
Equation 14: Matrix representation of \( C_{\text{VOI}} \).

To review, \( c \) and \( A \) are the signal intensity changes in the time density curves of the vessel of interest and the input function, respectively. The residual, \( b \), is what needs to be solved. This can be done as \( b = A^{-1} \cdot c \). To obtain \( A^{-1} \), a singular value decomposition (SVD) technique can be applied that converts the inverse matrix into the product of three matrices: \( A^{-1} = V \cdot W \cdot U^T \) where \( W \) is a diagonal matrix, and \( V \) and \( U^T \) are orthogonal and transpose orthogonal matrices, respectively. The main benefit of the SVD approach is that the diagonal elements of \( W \) are 0 or close to 0 because the rows in \( A \) are linear combinations of each other. This allows for elimination of noise (oscillating or meaningless in a biomedical modeling context, negative values for example) by setting values in \( W \) to zero below a threshold, typically 20\% \(^{80} \). By doing so, the resulting \( b \) (residual) is the best possible solution in a least squared sense for the vector norm of \( |A \cdot b - c| \). The maximum height (highest probability) of the just solved residual function (probability density function of transit times representing blood flow, \( F_t \)) is the relative flow rate \(^{81, 82} \).

Pulmonary blood volume (PBV) was estimated from the area under the time-concentration curve normalized to the area under the arterial input function (AIF, typically a feeding vessel of the vasculature of interest) curve \(^{80} \). Mean transit time
(MTT) was calculated by the blood volume divided by the flow rate (central volume theorem)\cite{80}.

The blood flow results presented at this point is a relative measurement. In the following sections (3.3.2.3 X-ray DSA Derived Flow Metrics), work was done to calibrate the relative measurement to become an absolute measurement (mL/min). Because of the superposition nature of planar imaging and the normalization to the AIF area under the curve, PBV remained a relative measurement.

In this micro-injector study, the pulmonary artery root was used as the arterial input function. Coefficient of variations were calculated for PBV, PBF, and MTT from the six injections for each ROI. The coefficient of variation (Cv) was measured as:

\[
C_v = \frac{\sigma}{\mu}
\]

\textit{Equation 15: Coefficient of variation}

where \(\sigma\) is the standard deviation and \(\mu\) is the mean. In addition, p-values (one-way ANOVA) were determined by comparing the MTT between the various injection volumes within the same ROIs.
Figure 24

(a) left pulmonary artery
i) 50 \( \mu \)L
ii) 100 \( \mu \)L
iii) 150 \( \mu \)L

(b) right lung tissue

(c) left ventricle

(d) aorta
Figure 24: Time density curves of the rat in the left pulmonary artery (a), right lung parenchyma (b), left ventricle (c), and aorta (d). The regions of interest used are shown in Figure 7. As expected, there was a shift in the curves as the contrast moves further along the vasculature. Note the repeatability and scalability of the injector with injection volumes starting from 50 µL (i), increasing to 100 (ii), and then 150 µL (iii), and then going from 150 µL back down to 100, and finally 50 µL.
Table 4: Pulmonary blood volume (PBV), pulmonary blood flow (PBF), and mean transit time (MTT) averages, ±1 standard deviations, and coefficients of variation (Cv) of all injection volumes (50, 100, and 150 µL pairs) for each ROI in Figure 23. The PBV, PBF, and MTT values were calculated using SVD of the time density curves in Figure 26. The pulmonary artery root was used as the arterial input function. Note how the coefficients of variation are all < 22%, with most being between 0.04-8.2%. In addition, the change in MTTs between the same ROIs for each injection volume was statistically insignificant (all p-values ≥ 0.816). The SVD based flow metrics here are relative measurements. Work was done to calibrate the relative measures to absolute measurements as seen in 3.3.2.3 X-ray DSA Derived Flow Metrics.
<table>
<thead>
<tr>
<th>values: avg. ± 1 S.D.</th>
<th>PBV (a.u.)</th>
<th>C_v (%)</th>
<th>PBF (a.u.)</th>
<th>C_v (%)</th>
<th>MTT (s)</th>
<th>C_v (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>50µL inj.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left pulmonary artery</td>
<td>0.86±0.012</td>
<td>1.5</td>
<td>1.88±0.12</td>
<td>6.4</td>
<td>0.47±0.030</td>
<td>6.4</td>
</tr>
<tr>
<td>Right lung tissue</td>
<td>1.01±0.078</td>
<td>7.7</td>
<td>1.09±0.05</td>
<td>4.8</td>
<td>0.96±0.025</td>
<td>2.6</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>1.61±0.89</td>
<td>5.5</td>
<td>1.58±0.13</td>
<td>7.9</td>
<td>1.04±0.140</td>
<td>13.5</td>
</tr>
<tr>
<td>Aorta</td>
<td>0.71±0.034</td>
<td>4.7</td>
<td>0.77±0.13</td>
<td>17.1</td>
<td>0.95±0.207</td>
<td>21.9</td>
</tr>
<tr>
<td><strong>100µL inj.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left pulmonary artery</td>
<td>1.00±0.005</td>
<td>0.5</td>
<td>2.36±0.042</td>
<td>1.8</td>
<td>0.44±0.014</td>
<td>3.3</td>
</tr>
<tr>
<td>Right lung tissue</td>
<td>1.02±0.003</td>
<td>0.3</td>
<td>1.20±0.022</td>
<td>1.9</td>
<td>0.89±0.010</td>
<td>1.2</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>1.82±0.110</td>
<td>6.0</td>
<td>1.84±0.032</td>
<td>1.7</td>
<td>1.00±0.079</td>
<td>7.9</td>
</tr>
<tr>
<td>Aorta</td>
<td>0.69±0.022</td>
<td>3.1</td>
<td>0.76±0.0002</td>
<td>0.04</td>
<td>0.92±0.029</td>
<td>3.1</td>
</tr>
<tr>
<td><strong>150µL inj.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left pulmonary artery</td>
<td>1.05±0.025</td>
<td>2.4</td>
<td>2.38±0.196</td>
<td>8.2</td>
<td>0.46±0.053</td>
<td>11.5</td>
</tr>
<tr>
<td>Right lung tissue</td>
<td>1.02±0.013</td>
<td>1.2</td>
<td>1.22±0.032</td>
<td>2.6</td>
<td>0.87±0.048</td>
<td>5.5</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>1.85±0.004</td>
<td>0.2</td>
<td>1.76±0.017</td>
<td>0.9</td>
<td>1.06±0.013</td>
<td>1.2</td>
</tr>
<tr>
<td>Aorta</td>
<td>0.69±0.017</td>
<td>2.5</td>
<td>0.83±0.043</td>
<td>5.2</td>
<td>0.84±0.022</td>
<td>2.6</td>
</tr>
</tbody>
</table>
Time-density curves of the left pulmonary artery, right lung parenchyma, left ventricle, and aorta seen in Figure 23 show that the changes in vessel enhancement for injection volumes ranging from 50-150 µL (Figure 26). Note the almost identical overlap of the time-density curves for the same pairs of injection volumes when systematically increasing the injection volume from 50 µL to 150 µL and then back down from 150 µL to 50 µL. The coefficient of variation measured from the SVD-derived PBV, PBF, and MTTs (Table 4) showed little change with the three pairs of injection volumes. The greatest Cv was < 22% with the majority being between 0.04-8.2%. There was no statistically significant difference in the MTT between injection volumes as measured by one-way ANOVAs: p-value = 0.817 (left pulmonary artery) and 0.816 (right lung parenchyma, left ventricle, and aorta). The peak gray intensity value enhancement of the time-density curve scaled linearly with increasing injection volumes.

In the subsequent in-vivo tests, the changes in vessel enhancement were found are due only to physiological effects, not injector-related parameters. Larger injection volumes in the left pulmonary artery, right lung parenchyma, left ventricle, and aorta (Figure 23) created only amplitude shifts in the time-density plots; no shift was seen in the transit time (Figure 26). The flow metrics (relative measurements) derived from the SVD technique agree with this, as seen in the low coefficient of variation values (Table 4). There was no statistically significant difference (all p-values ≥ 0.816) in the MTT as measured by one-way ANOVAs. The MTT was independent of the injection volumes (50, 100, and 150 µL).

Additional imaging studies were performed to demonstrate synchronization across the phases of the cardiac cycle. In the first study, imaging was performed at different intervals (systole, diastole, and diastasis) with contrast injections at the same point (QRS) in the cardiac cycle.
Figure 25: Multiple images acquired at fixed times in the rat during (a) ventricular systole, (b) diastole, and (c) diastasis with single 100 µL contrast injection initiated at the QRS. The contrast bolus transit in the circulation can be followed across time. Note how the enhancement of the left ventricle (dashed arrow) diminishes over time, while the aorta becomes more enhanced (solid arrow).

The response time of the injector is a critical element of its performance. The R-R interval of a rat is ~150 ms and the readout for the camera is ~100 ms. While each radiographic exposure is ~10 ms, the camera readout might limit the temporal resolution to 100 ms. Yet the reproducibility of the injection allowed three different injections at the same point in the cardiac cycle with the 10 ms radiographic exposure adjusted to
capture three time points separated by as little as 25 ms. Thus, row 1-a (Figure 25) shows the right ventricle for injection 1 at the QRS (systole). Row 1-b shows the right ventricle 50 ms (diastole) after the QRS, for the second injection. And row 1-c shows the right ventricle 75 ms (diastasis) into the R-R interval for the third injection. The reproducibility of the injector and the biological sequence allowed viewing the physiology at temporal resolution of 25 ms—even though the camera readout is 100 ms. Changes in contrast enhancement were seen in the left ventricle (dashed arrow) and aorta (solid arrow) when the time of injection (at the QRS) was kept constant and images were acquired at systole, diastole, and diastasis.

In the second study, instead of injecting during a fixed time point in the cardiac cycle, contrast injections were first made at the QRS, and then 30, 50, 60, and 75% of the R-R interval after the QRS with imaging at a fixed time point, the QRS. The anatomic target was the coronary arteries.

![Figure 26: Coronary artery imaging (arrows) of the rat at the same time point in the cardiac cycle, at the QRS, with a 150 µL contrast injection at the QRS, 30, 50, 60, and 75% of the R-R interval after the QRS (left to right).](image)

Figure 26 shows the impact of timing in one of the most critical imaging studies, i.e. demonstration of the coronary arteries. In this experiment, a 150 µL injection was
made at the QRS. Subtraction was performed on the next cardiac cycle at the QRS. The same experiment was repeated with delays between the QRS and injection ranging from 30-75% of the R-R interval. The injection at the QRS occurs at systole during which the aortic valves are open and allows visualization of the coronary arteries. During this period, the contrast can flow retrograde and then enter the coronary arteries upon normal flow. At all other times, the retrograde flow is not possible, so the left ventricle (LV) does not fill. Timing differences of the injection was < 100 ms. This made the difference between seeing the coronaries (@ 0% delay) and seeing only the ascending aorta.

The precision in timing of the injections has an advantage for imaging the vessels in the heart. The custom LabVIEW application allowed placement of the contrast injection at specific points in the cardiac and ventilatory cycles, which is critical in imaging rapid physiologic changes such as the one seen in Figure 25 and Figure 26. Figure 25 shows injection repeatability while adjusting the x-ray exposure to delineate functional flow changes with 25 ms temporal resolution. Figure 26 shows that the response time of the injector is sufficient to allow for injections with such precision that the left ventricle was filled during systole when the aortic valve is open and allows for visualization of the coronary arteries upon resuming normal blood flow, demonstrating what is believed to be the first coronary DSA images in a rat.

Finally, DSA images of the mouse kidney were acquired to demonstrate the utility of the micro-injector in the smaller (25 g) model. The target organ was the kidney with injection via the iliac artery.
Figure 27: Mouse renal DSA at 46 μm projection resolution. Note the vasculature of the renal cortex and adrenals (white arrows) and the enhancement of the renal arteries (black arrows). The injection catheter was just distal to the left renal artery to allow enhancement of both kidneys. Note delayed enhancement of the left renal cortex due to the arterial offset (3.5 mm) of right and left renal arteries.

The mouse @ 25 grams is yet 10-times smaller than the rat with a heart rate roughly 3-times that of a rat, which poses even greater challenges for the injector. Figure 27 shows a DSA image @ 46 μm resolution of the renal vessels of a live mouse acquired with a 150 ms (20 μL) bolus injected at the QRS. Images were acquired at end systole on every heartbeat (R-R interval of 136 ms). The catheter was placed in the iliac artery so that the tip was at the level just distal to the left renal artery. During the first heartbeat, the right renal artery fills some of the feeding vessels to the renal cortex. The second heartbeat demonstrates more complete filling of the right renal vasculature. The vasculature in the left kidney fills later due to the relative displacement (3.5 mm) of the left and right renal arteries.

Placement of the catheter to enhance only the vessels of interest is critical in producing a quality subtraction angiogram, as seen in Figure 27. Contrast can easily flow into the iliolumbar artery (just distal to the left renal artery) because it has a larger...
diameter. This was especially true for the mouse imaging, where the distance between the left renal and iliolumbar arteries connect to the descending aorta at very close proximity (1.4 mm). Careful placement of the injection catheter allowed visualization of the renal arteries, cortex, and adrenal glands. This opens the door to renal blood flow imaging experiments.

To our knowledge, this study represents the first demonstration of a computer-controlled injector for vascular imaging in the small animal. The injector described supports response times, injection rates, and injection volumes appropriate for both rats and mice. For the mouse, the injection rate is limited to 0.2 µL/ms by the bore of the catheter. The larger catheter used in the rat allowed variation of the injection rate linearly from 0.1 to 1.0 µL/ms by adjusting the driving pressure. Injection volumes of 100-150 µL (0.83-1.25% of the blood volume) provided reproducible opacification of the cardio-pulmonary vasculature with minimal impact on the physiology. Injection volumes of 20 µL (1% of the blood volume) allowed visualization of the renal vasculature of the mouse. Changes in the time-density curve shapes of selected regions of interest were due primarily to physiological changes, independent of micro-injector parameters.

The linear reproducibility of the injection volumes and flow rates, and the ability to inject at specific time points during the cardiac cycle opens doors to experiments not previously possible. The utility of this system can be found in visualizing and quantifying real-time changes in blood flow in a variety of organ systems, e.g. cardio-pulmonary and renal systems. The micro-injector is an important component in designing an optimal system for small animal digital subtraction angiography where the spatial and temporal resolutions require a unique approach. The injector has already been applied to a number of x-ray and MRI studies for vasculature imaging, perfusion, and flow measurements.
3.3 Complimentary/Quantitative Physiological Functional Measurements

This section describes the functional measurements of physiology in the small animal from the monitoring and ventilation systems, and four methods to quantify blood flow.

3.3.1 Small Animal Monitoring and Ventilation Systems

Like a clinical operating room, a series of physiologic signals are monitored while the subject is under ventilated anesthesia. The small animal monitoring system consists of three components: transducers, signal conditioners, and software. The monitoring transducers include biological and system signals. Typical biological signals include ECG, body temperature, inspiration pressure and flow, and blood pressure. System signals include TTL pulses that identify when x-ray exposures are being made and injection of contrast agent. These signals are fed into hardware amplifiers and filters (Lablin V, Coulbourn Instruments, Allentown, PA) for signal conditioning and processing (e.g. identifying the QRS in the ECG). These signals are then fed into LabVIEW software through a 16-channel, 500Hz, high precision A/D board (PCI-6025E, National Instruments, Austin, TX). The software program can read, display, record, and interpret physiologic and system signals. The signals are continuously collected and displayed for the duration of the experiment. These signals are also used to control the image gating described in the Biological Pulse Sequence section of the System Integration chapter. The monitoring software (Figure 28) displays traces in three forms: real time (a), snapshots (b), and longitudinal trends (c).
Figure 28: Biological and system signals monitor. Screen (a) displays real time signal traces, (b) displays signal snapshot over user defined window, and (c) graphs longitudinal trends.

Real time traces (a) are used to see immediate effects of signal filtering. This is primarily for the rodent ECG trace because the mouse heart beats at 600 times a minute, 10x that of a human, and has a small signal amplitude (in the mV) while being located in an electrical noise prone environment. Data snapshots (b) are displayed over a user-defined window. This allows the experimenter to see interactions between physiological signals and system timed events (x-ray exposure, contrast injection, etc.). Longitudinal trends (c), such as heart rate and body temperature, are recorded every five seconds. Based on the trends, levels of anesthetic and heating are adjusted. This monitoring hardware and software have been ported for use at the MRI and PET imaging stations. Further detail of the code can be found in the Appendix.
A constant volume ventilator, similar to \cite{85, 86}, was designed explicitly for use in the x-ray environment (Figure 29). Modifications to the above references included support for vaporized anesthetic, inhalation and exhalation gas sampling ports, more responsive pressure and flow sensors, quick disconnects of all control cables and gas lines for easier maintenance, and overall compactness. For experiments where a constant pressure ventilator is required, the system developed in \cite{87, 88} is also available and interfaces with the same software as the constant volume ventilator described in the section above.

![Figure 29: Constant volume ventilator. The primary components include inspiration and expiration solenoids, pneumotach, flow and pressure sensors, and a platform with variable height adjustment to accommodate mice and rats.](image)

A custom LabVIEW program has been developed to control the constant pressure and flow ventilators (Figure 30). Timing calculation and signal generation occur on the counter/timer board level (PCI-6602 National Instruments, Austin, TX). This not
only provides instant response to parameter adjustments, but also allows operation of the ventilator even if the host computer fails. In addition to supporting the most basic ventilating functions of inspiration and expiration, the software enables the user to suspend breathing at full inspiration or end expiration. A direct application of apnea is in eliminating ventilatory motion artifacts. Other applications may include studies comparing changes in pulmonary blood flow at full inspiration and at full expiration. The ventilator software interface also sends TTL pulses for every breath to the x-ray sequencer for use in ventilation-gated imaging. This software has been adapted for use in the MRI imaging stations of the CIVM. An additional feature is the capacity for hyperpolarized gas delivery to image the lung air space. Further detail of the code can be found in the Appendix.

Figure 30: Ventilator control interface where breathing rate, inspiration, breath hold, and exhalation durations are set. Any of those values can be adjusted in real time.
3.3.2 Quantitative Blood Flow Measurements

The use of small animals in research has grown in recent years because of an increasing interest in rodent drug and disease models. Cardio-pulmonary blood flow is an important indicator of physiologic state/response to drug/disease stimulus. Current methods for measuring cardio-pulmonary blood flow include: Fick, thermodilution, magnetic flowmetry, microspheres, Doppler ultrasound, MRI, CT, and PET. The Fick method is the gold standard for measuring cardiac output in absolute units (mL/min). However, the Fick method is a global, whole body measurement (non-regional) and is limited to a small number of sample acquisitions since it requires blood withdrawals of 1-2mL for each measurement. Multiple measurements can be made in the same rodent using thermodilution, an advantage over the Fick method, but the reported values are relative measurements. Magnetic flowmetry can produce absolute units (when properly calibrated) but is very invasive (a midline sternotomy is commonly performed to gain access to the aorta for cardiac output measurements). In addition, internal vessels of the lung are out of reach for the probe placement because the pulmonary vessel branches are within the lung. The use of microsphere can produce regional blood flow information, a benefit over previous methods, but the results are relative values (commonly in concentration). In addition, only a few acquisitions can be performed in the same animal and this technique requires animal sacrifice. Doppler ultrasound, MRI, CT, and PET offer non-invasive solutions that allow for multiple measurements in the same animal and vascular visualization. Ultrasound offers high temporal sampling but is limited in measuring parenchymal blood flow because of the air in the lungs. In addition, the results are in terms of velocity (cm/min) rather then flow (mL/min). This characteristic makes the Doppler ultrasound measurements dependent on vessel size. CT and MRI are both used clinically. CT perfusion is a very active area of research.
but scaling CT to the resolution necessary for small animals requires much longer scan times (10’s of seconds to minutes) which is too long to capture rapid, acute changes in blood flow. Several different methods have been developed for perfusion MRI based on flow or the use of injectable contrast agents. But these methods are also challenging when translating to the spatial and temporal resolution required for small animals and calibration can be particularly problematic. The system described here, a small animal x-ray digital subtraction angiography (DSA) system, is a solution to these shortcomings. DSA is a particularly appealing approach because it is easy to use, and it can capture rapid physiological changes on a heart beat-to-beat basis. The anatomical imaging from DSA can be used to derive blood flow metrics using a non-parametric deconvolution technique. This blood flow calculation is a relative measurement specific to each animal. For single small animal studies, this may suffice, but in n > 1 studies, an absolute measurement is required. The focus of this work is mapping the DSA relative flow metric to an absolute metric through physiologic based measurements—the Fick and thermodilution techniques. The Fick method is the gold-standard for finding cardiac output and is a ratio of the total blood oxygen uptake by the peripheral tissues to the arterial-venous blood oxygen concentration difference. The thermodilution technique is an indicator-dilution method where the regional blood flow is measured by noting temperatures changes induced at specific points through the injection of a cold liquid. A chain of calibrations was performed linking Fick to thermodilution to DSA. The thermodilution method provided a particularly unique opportunity to calibrate the Fick and DSA because the x-ray contrast agent also served as the thermodilution injectate. This allowed multiple, simultaneous measurements of cardiac output for statistical analysis while providing vascular visualization and calibration of the DSA based flow metric to an absolute measurement. The resulting calibrated DSA solution can perform: 1) minimally invasive in vivo blood flow
measurements, 2) multiple measurements in the same animal in a rapid succession (every 30 seconds—a substantial improvement over singular measurements that require minutes to acquire by the Fick method), 3) very high resolution (up to 46 micron) vascular visualization, 4) quantitative blood flow measurements in absolute metrics (mL/min instead of arbitrary units or velocity) for where the correction for attenuation derived from the vessel thickness can be applied and relative blood volume dynamics from discrete ROIs, and 5) relative mean transit time dynamics on a pixel-by-pixel basis (100 µm x 100 µm). The end results are 1) anatomical vessel time course images showing the contrast agent flowing through the vasculature, 2) blood flow information of the live rat cardio-pulmonary system in absolute units and relative blood volume information at discrete ROIs of enhanced blood vessels, and 3) colormaps of relative transit time dynamics. The sections below describe the calibration process from Fick to thermodilution to x-ray DSA. The Fick’s results had <14% coefficient of variation between all eight rats and had similar measures as in another study. This provided a platform from which the thermodilution and x-ray DSA could be calibrated. Catheter placements for a mixed venous blood sample (for Fick’s method) and temperature measurement (for thermodilution) were critical. In particular, separate calibration factors were found based upon the placement of the distal thermodilution temperature sensor. The separate thermodilution calibration factors were found to have little impact on the DSA calibration factors (percent difference < 9.5%). So, the DSA calibration of $18.90 \times CO_{\text{DSA}} = CO_{\text{Fick}}$ could be used solely in future studies. The vessel thickness correction and it verification allow for extendibility of the SVD method across a range of rat sizes and vessel thicknesses. Thus, the calibration factor results can be used for future x-ray DSA and thermodilution studies to measure drug or disease modulated cardiac output and pulmonary blood flow dynamics.
3.3.2.1 Fick’s Cardiac Output

Three catheters (Figure 31) were placed in eight male rats (251-287g, Fischer-344) for x-ray contrast agent injection (right jugular vein at the root of the superior vena cava), temperature measurement (left common carotid artery at the level of the aortic arch), and blood sampling (left femoral vein with the sampling site in the right ventricle).
Figure 31
Figure 31: Schematic of the rat heart showing the catheter locations for x-ray DSA contrast delivery (injection catheter), thermodilution temperature measurement (distal temperature sensor and shared use of the DSA injection catheter), and Fick blood sampling (mixed venous blood sampling catheter and shared use of the thermodilution distal temperature sensor). The catheter locations were confirmed using x-ray guidance. The injection catheter tip was at the level of the right atrium and used the right jugular vein as the access point. The distal temperature sensor catheter was inserted via the left common carotid artery so that the catheter tip was immediately superior to where the carotid artery branches from the aortic arch. The thermocouple wire from the distal temperature sensor catheter was advanced from this location. Depending on each specific rat’s aortic arch variation, the sensor probe tip (represented by the dotted lines) was placed either immediately superior to the aortic valve in the ascending aorta or in the descending aorta. The access point for the mixed venous blood sampling catheter was the left femoral vein. This catheter was then sent into the right side of the heart via the inferior vena cava. This custom catheter had two curves near the tip. A wire was placed inside this catheter while it was advanced to the heart. The wire helped in guiding the catheter and rebending the two pre-shaped curves to be straight for easier catheter advancement. When the tip of the catheter was just outside of the right atrium, the wire was pulled out so that the pre-shaped curves hooked the catheter tip into the right ventricle.
The Fick’s CO method was used as the reference to calibrate the thermodilution based CO scaling constant. The Fick’s method relies on the observation that the total blood oxygen uptake by peripheral tissues is equal to the product of the blood flow to the peripheral tissues and the arterial-venous oxygen concentration difference. Fick’s CO is mathematically expressed as (Equation 16):

$$\text{cardiac output} = \frac{\dot{V}_{O_2}}{C_aO_2 - C_vO_2}$$

Equation 16: Fick’s cardiac output

where

$$\dot{V}_{O_2} = \text{oxygen uptake} = \text{minute ventilation volume} \times \left( \%O_2 \text{ concentration}_{\text{Inhale}} - \%O_2 \text{ concentration}_{\text{Exhale}} \right)$$

$C_aO_2 = \text{arterial blood oxygen concentration}$

$C_vO_2 = \text{venous blood oxygen concentration}$

Two catheters were used to obtain the arterial and venous blood samples. Mylar collection bags connected to the small animal ventilator obtained samples of inspired and expired breathing air. More details of the blood and gas sampling are described below.

After acquiring 10 data sets of thermodilution and x-ray based CO measurements (methods described in the sections below), ~500µL of venous blood was drawn to measure $C_vO_2$. The venous blood sampling catheter (PE-50) was inserted in the left femoral vein. From there, the catheter was sent up the inferior vena cava to place the catheter blood sampling tip in the right ventricle. The placement of this catheter was a non-trivial task and was essential to obtaining an accurate mixed venous blood gas sample that accurately represented the O₂ consumption of the entire body. A vena cava blood sample would represent only a portion of the body, and a right atrium sample would not have complete venous mixing. The position of the catheter was verified using
x-ray imaging. Immediately after venous blood sampling, the distal temperature sensor catheter was used to withdraw ~500µL of arterial blood to measure $CaO_2$ via the left common carotid artery from the aortic arch, where the oxygen concentration is the highest. The arterial blood sampling catheter served the dual purposes of acquiring arterial blood samples and of measuring temperature, this sensor function is described in the thermodilution section. The catheter insertion procedure was guided by real-time blood pressure measurements and verified by x-ray imaging. Both blood samples were drawn into pre-heparinzed syringes and placed in an ice chest. The blood gas was analyzed (682 CO-Oximeter, Instrumentation Laboratory, Lexington, MA) for its arterial ($CaO_2$) and venous ($CvO_2$) oxygen content within 30 minutes of when the samples were drawn. During the blood sampling, the inhaled and exhaled breathing air was captured in gas sample bags for calculating $O_2$ uptake ($\dot{V}O_2$). The oxygen concentration in the breathing and exhaled air was then measured using a gas chromatographer (3800 Gas Chromatographer, Varian Inc., Palo Alto, CA). The measurements were corrected for standard temperature and pressure dry. Minute ventilation volume was measured using a spirometer. The ventilator was gas tight and the rats breathed air that was 20.9 ± 0.1% O2 and <0.6% CO2.

The Fick’s CO average and standard deviation for the rats were calculated. To measure the intervariability between the rats, the coefficient of variation (Equation 17) was calculated as:

$$\text{coefficient of variation} = C_v = \frac{\sigma}{\mu}$$

Equation 17: Coefficient of variation

where $\sigma$ is the standard deviation and $\mu$ is the mean. Using the average Fick’s CO measurement, the scaling constants in thermodilution were found, as described below.
Characteristics of the rats and results of the CO measurements can be found in Table 5. The eight rats used in this study were similar in body weight (268.5±10.7g, 4.0% CV) and heart rate (374.5±19.6BPM, 5.2% CV). The average, standard deviation, and coefficient of variation in both tables are highlighted in bold to show the relationship of each physiologic measurement.
Table 5: Measured and calculated values of CO using Fick’s method, thermodilution, and x-ray DSA arranged in two groups—the distal temperature sensor in the descending (DA) and ascending aorta (AA). Of interest are the statistical values (average, standard deviation, and coefficient of variation values are in bold) for body weight, heart rate, Fick’s cardiac output value, stroke volume, thermodilution ($K_{Thermo}$), and x-ray DSA ($K_{DSA}$) scaling constants. The rats had very similar body weights and heart rates. This allowed CO to be measured on anatomically and physiologically similar animals. The Fick’s CO had a $C_v$ (13.9%) that allowed for accurate mapping of thermodilution and x-ray DSA based CO scaling constants. For the DA case, the $K_{Thermo}$ and $K_{DSA}$ scaling constants were $0.44 \pm 0.04$ a.u., 9.8% $C_v$ and $40.48 \pm 2.63$ a.u., 6.5% $C_v$, respectively. The AA case resulted in $K_{Thermo}$ and $K_{DSA}$ scaling constants of $0.89 \pm 0.19$ a.u., 20.8% $C_v$ and $22.33 \pm 1.94$ a.u., 8.7% $C_v$, respectively. Ten thermodilution samples and ten x-ray DSA sequences were acquired for each rat.
<table>
<thead>
<tr>
<th>Distal Temp. Sensor:</th>
<th>In Descending Aorta (DA)</th>
<th>In Ascending Aorta (AA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>268</td>
<td>273</td>
</tr>
<tr>
<td>Heart rate (BPM)</td>
<td>359</td>
<td>382</td>
</tr>
<tr>
<td>Fick’s Method CO (ml/min)</td>
<td>Avg.</td>
<td>Std.</td>
</tr>
<tr>
<td></td>
<td>37.15</td>
<td>35.03</td>
</tr>
</tbody>
</table>

Thermodilution CO (a.u.), precalibrated

| Avg. | 76.4 | 82.5 | 64.1 | 78.7 | 39.1 | 46.9 | 46.2 | 53.1 |
| Stdev. | 2.8 | 3.6 | 3.5 | 2.5 | 5.0 | 4.2 | 2.1 | 5.4 |
| CV (%) | 3.65 | 4.35 | 5.51 | 3.20 | 12.89 | 9.03 | 4.55 | 10.15 |

| Avg. | Stdev. | CV |
| K<sub>Thermo</sub> | 0.49 | 0.42 | 0.47 | 0.39 | 0.44 | 0.04 | 9.8 | 1.16 | 0.90 | 0.78 | 0.74 | 0.89 | 0.19 | 20.8 |

X-ray DSA CO (a.u.), precalibrated

| Avg. | 1.79 | 1.95 | 1.61 | 2.12 | 1.93 | 2.02 | 1.88 | 2.51 |
| Stdev. | 0.40 | 0.31 | 0.36 | 0.30 | 0.28 | 0.20 | 0.19 | 0.49 |

| Avg. | Stdev. | CV |
| K<sub>DSA</sub> | 42.76 | 42.34 | 39.79 | 37.06 | 40.48 | 2.63 | 6.5 | 20.30 | 23.25 | 24.57 | 21.18 | 22.33 | 1.94 | 8.7 |
Using the Fick’s method, the average CO between the eight rats was $37.0 \pm 5.1$ ml/min (13.9% $C_v$). In previous work using only the Fick method, CO measurements in the same animal yielded < 5% variation between measurements $^{99,100}$. The variation could be attributed to the comparison between animals versus within the same rat and the increased complexity of this study over previous work by the inclusion of thermodilution and DSA. In this experiment, ~1 mL total of arterial and venous blood was drawn. This is < 10% of the total blood volume. However if a second measurement were made, the total blood withdrawn (~2 mL) would constitute ≥ 15% of the total blood volume in the rat and could pose a physiologic impact. In addition, the process of acquiring the arterial-venous blood samples and the inspiration-expiration breathing gases requires a few minutes. So acute, rapid changes in the blood flow physiology may not be captured because of the insufficient temporal resolution. These limitation are not present for thermodilution and x-ray DSA based measurements, thus allowing multiple blood flow measurements to be acquired within a short amount of time (every ~30 seconds). For the live animal study, the rat strain, sex, body weight, physiologic state, and anesthetic type (or conscious state) can significantly impact the CO $^{6,7,9,101-103}$. The cardiac outputs experienced in the rat can range from 25 ml/min in a deeply anesthetized rat $^{93}$ to 50 ml/min in a conscious rat $^5$. Using the gold standard method in this study produced similar CO values ($37.03 \pm 5.14$ ml/min, 13.87% $C_v$) as another study ($43 \pm 10$ mL/min) that used microspheres in anesthetized male Fischer-344 rats of similar body weight $^5$. The variation in the Fick’s CO method between the eight rats in this study was <10%. Based on these foundations, the thermodilution and DSA techniques were calibrated (described in the following sections).
3.3.2.2 A Novel Thermodilution Technique

The thermodilution technique is an indicator-dilution method where an injectate induced temperature difference between two points in a region of flow is a function of the temperature change, the volume of injectate, and the heat characteristics of the injectate and blood. Mathematically, this is expressed by the Stewart-Hamilton equation (Equation 18)\textsuperscript{94, 95, 97}:

\[
\text{cardiac output} = \frac{K_{\text{thermo}} V_i (T_i - T_b) \rho_i c_i}{\rho_b c_b \int \Delta T(t) dt}
\]

Equation 18: Stewart-Hamilton equation for thermodilution

where

- \( K_{\text{thermo}} \) = scaling constant
- \( \rho_i c_s \) = density (g/cm\(^3\)) and specific heat (J/(g \cdot ^\circ C)) of injectate \( x=i \) or blood \( x=b \)
- \( V_i \) = injectate volume (ml)
- \( T_i \) = initial temperature of injectate \( x=i \) or blood \( x=b \) (\(^\circ C\))
- \( \Delta T \) = temperature change between injectate and distal temperature sensors (\(^\circ C\))

The thermodilution technique is routinely performed in the clinical setting to measure CO using a Swan-Ganz catheter\textsuperscript{98}. This special catheter is multi-lumened along its length to allow injection, temperature measurement, and sampling. In the clinical setting, room temperature or chilled saline is injected in the right atrium and the temperature is measured at the tip of the catheter in the pulmonary artery. This approach was unique because warmed x-ray contrast agent (Isovue 370, 370mg iodine/mL, Bracco Diagnostics, Princeton, NJ) was used, instead of chilled saline, to acquire x-ray DSA images and thermodilution curves at the same time. Another important design feature was that the distal temperature sensor was a separate catheter that was placed in the aortic arch via the left common carotid artery to allow adequate time for injectate-blood mixing because rats have a rapid heart rate (~375 BPM). In addition, the aorta was
easier to access than the pulmonary artery given the tremendous scaling in size between man (80,000 g) and rat (250 g). The two catheters (injection and distal temperature sensor) are described below.

The contrast injection catheter (Figure 34a) was made up of a series of cascading diameter tube segments (3.75 cm, PE-50 at the tip to 1.25 cm, PE-190 to 6.5 cm, PE-240 at the end) that ensured the catheter could fit in the jugular vein while maintaining a large enough lumen for contrast agent delivery.
Figure 32

a) injection catheter
- thermocouple sensor port
- contrast injection port
- coaxial heating tube
- injection catheter
- right jugular vein
- thermocouple sensor tip location

b) distal temperature sensor catheter
- flush syringe
- right carotid artery
- thermocouple sensor port
- thermocouple wire
Figure 32: Thermodilution and x-ray DSA catheters. The injection catheter (a) delivered warmed contrast agent, and the distal temperature sensor catheter (b) measured the change in temperature downstream from the injection site. The injection catheter (a) was used for both thermodilution and x-ray DSA based CO measurements. This catheter was constructed from a small diameter tube with a larger surrounding heating tube, with ports for contrast injection and temperature measurement. The injection catheter was inserted into the right jugular vein such that the tip of the catheter was at the level of the right atrium. Contrast agent was then delivered using a micro-injector through a branch in the Y-tube that led to the injection port. A heating tube was coaxially wrapped around the contrast carrying catheter. An external, heated pump system ensured the injectate temperature was maintained at ~50°C. A thermocouple inserted through the sensor port (the other branch in the Y-tube) provided the temperature readings for the injectate. The measuring tip of the sensor was located in the center of the contrast catheter surrounded by the coaxial heating tube. The distal temperature sensor catheter (b) was inserted through the left common carotid artery such that the tip was placed just superior to the carotid artery branch from the aorta. The thermocouple wire was then deployed under x-ray guidance from the catheter for direct temperature change readings and repeatable positioning. Depending on each specific rat’s unique aortic arch variation, the sensor probe tip (represented by the dotted lines in Figure 31) was either in the ascending aorta or in the descending aorta. A flushing syringe cleared blood clots at the catheter tip to allow placement of the thermocouple wire. This same catheter was also used for obtaining the arterial blood sample for the Fick’s CO measurement.
In addition, the injection catheter had a heating tube coaxially wrapped around the contrast carrying catheter and ports for temperature measurement and contrast injection. The tubing segments (PE-190 and PE-240) heated by the coaxial heat tube held the majority (>90%) of the injectate volume. The remaining catheter length was in the rat and thus at body temperature. A temperature controlled water bath and a peristaltic pump were used to circulate warm water around the injection catheter via the coaxial heating tube. The coaxial heating tube was constructed from a pair of hemostasis valves with side ports (Part # 80390, Qosina, Edgewood, NY). The heating assembly ensured the injectate temperature was maintained at 50±0.1°C. Because the injectate volume is small (100µL), the net temperature increase in the animal is < 0.4°C (Figure 35) and so the warmed contrast agent minimally impacts the physiology. A thermocouple (T-type with 0.005 seconds time constant, IT-23, Physitemp, Clifton, NJ) inserted through the sensor port (2 Fr Touhy Borst adapter, Part #: S19244, Qosina, Edgewood, NY) provided the temperature readings for the injectate. This thermocouple was chosen because of its small size (0.23 mm diameter sensor tip and ~0.15 mm diameter lead wires) and fast response time (time constant = 0.005 sec). The sensor was in the contrast catheter and the measuring tip was in the center of the contrast catheter surrounded by the coaxial heating tube. The small diameter (0.23 mm) of the thermocouple minimally impacted the flow in the PE-240 segment (1.67 mm inner diameter, 7.3X larger than the IT-23) of the injection catheter. The injectate temperature was measured and recorded for the entire thermodilution technique. This was an improvement over current clinical and small animal thermodilution systems, where only one temperature sample is made in an external beaker. In our approach, continuous temperature sampling and a closer temperature sensor placement to the injection site eliminated assumptions that no heat loss occurred between filling.
connecting, and dispensing the injectate as seen in other systems. Contrast agent was injected using the micro-injector through the injection port. The micro-injector used in this study delivered repeatable volumes (100±11µL) of injectate\textsuperscript{11, 42, 83, 84} and was shown in previous work to have little effect on blood flow physiology (statistically insignificant, \( p \geq 0.816 \), changes in blood flow transit time for a range of injection flow rates and volumes) \textsuperscript{42}.

The distal temperature sensor catheter (Figure 34b) was inserted through the left common carotid artery. The catheter tip (PE-10) was placed just superior to where the carotid artery branches from the aorta. This was the same catheter used to obtain the arterial blood sample for the Fick’s CO measurement. A 2 Fr Touhy Borst adapter, like the one used in the injection catheter, allowed for the thermocouple wire (IT-23) to pass out of the fluid-filled catheter for connection to the data acquisition module described below. This fluid-tight connection also allowed the thermocouple to be deployed (advanced or retracted) under x-ray guidance from the catheter when the Touhy Borst adapter was unlocked. The main challenge this design solves was guidance of a very delicate sensor (~0.15 mm diameter lead wires) through a length of winding vasculature (~15-20 mm) under high systolic pressure (~100-120 mmHg at systole). In addition, sending only the thermocouple into the aorta allowed for direct measurement of temperature changes with minimal disturbance of blood flow (0.23 mm diameter thermocouple in a ~4 mm aorta), and repeatable positioning of the thermocouple. Depending on each specific rat’s aortic arch variation, the sensor probe tip (represented by the dotted lines in Figure 31) was either just superior to the aortic valve in the ascending aorta or in the descending aorta, immediately after the arch. Because of the different measurement locations, the temperature change readings were significantly different, up to 48%. As such, the thermodilution cardiac output measurement results
were grouped into two categories based on the location of the distal temperature sensor—one for the sensor tip located in the descending aorta (DA, 4 rats) and the other category for the sensor tip located in the ascending aorta (AA, 4 rats). The DSA results were also grouped in this fashion because of the chain of calibrations linking the DSA to the thermodilution.

A flushing syringe cleared blood clots at the catheter tip. For both thermocouples, twelve readings were sampled every second and digitized at 24-bits using the USB-9211A (National Instruments, Austin, TX). The data acquisition and calculations of cardiac output were performed on in-house LabVIEW software (National Instruments). Of note is the calculation of the denominator term of the Stewart-Hamilton equation, $\int \Delta T(t) dt$ in eq. (Equation 18). The area under the temperature change curve (Figure 35, shaded region) was composed of two parts.
Figure 33
Figure 33: Simultaneous acquisition of x-ray DSA and thermodilution measurements resulted in DSA and temperature curves that follow similar time scales and shapes. The samples of DSA images (a-d) correspond to the selected points on the continuously acquired thermodilution temperature change between the aorta and the right atrium, and the DSA pixel intensity change over time (time density curve, TDC) in the aorta (circle in the DSA images). The figure shows the initial contrast injection with a. right ventricle filling; b. pulmonary venous phase and left ventricle filling with the beginnings of aortic output; c. left ventricular systole with full aortic output, which concluded with d. complete passing of the contrast agent. For the temperature change curve, the area in the shaded region is the denominator term of the Stewart-Hamilton equation that was used to calculate cardiac output. The shaded region was composed of two parts. The first part is the area under the temperature change curve that ranged from immediately after the contrast injection to 40% of the maximum temperature change in the descending limb. The second part was the area under an exponential curve that was fitted (circle-dotted curve) on the down slope starting from 40% of the maximum temperature change. This fit was based on the descending temperature range of 80% to 40% of the maximum temperature change value and was done to mitigate effects of indicator recirculation that resulted in a long, gradually decreasing temperature change tail.
The first part was the area under the temperature change curve that ranged from immediately after the contrast injection to 40% of the maximum temperature change in the descending limb of the graph. The second part was the area under an exponential curve that was fitted (circle-dotted curve) on the down slope starting from 40% of the maximum temperature change. This fit was based on the descending temperature range of 80% to 40% of the maximum temperature change value and was done to mitigate effects of indicator recirculation that resulted in a long, gradually decreasing temperature change tail (curve from 10 to 18 sec).

Ten thermodilution measurements, each separated by two minutes, were made for each rat. Statistics (average, standard deviation, and coefficient of variation) showing the CO measurements were found for each rat. The thermodilution scaling constant \(K_{Thermo}\) was then calculated (Equation 19) for each rat by:

\[
K_{Thermo} = \frac{\text{Fick's cardiac output}}{\text{Thermodilution cardiac output}}
\]

Equation 19: Thermodilution calibration factor

so that the relative thermodilution CO measurement matched that rat’s specific absolute Fick’s CO measurement. Statistics (average, standard deviation, and coefficient of variation) showing the interrelationship of the \(K_{Thermo}\) between the 4 rats in each DA and AA categories were also calculated. The temperature difference between the two measurement locations (distal temperature sensor in the descending or ascending aorta) directly translated into different measurements of thermodilution cardiac output and thus \(K_{Thermo}\). The pre-calibrated thermodilution based CO measurements averaged 75.43±7.96 a.u. (10.55% \(C_v\)) with the probe in the descending aorta, and 46.33±5.72 a.u. (12.34% \(C_v\)) with the probe in the ascending aorta. There was a 47.79% difference between the two measurement locations. Accordingly, the % difference between the DA
and AA $K_{\text{Thermo}}$ was large as well, 67.67% with an average $K_{\text{Thermo}}$ of 0.44±0.04, 9.8% $C_v$ and 0.89±0.19, 20.8% $C_v$ for the DA and AA rats, respectively. Thus, two categories were used to describe the cardiac output results for thermodilution. The Fick’s and x-ray based measurements were immune to location based CO measurements because the former is a global measurement and the latter is based on ROI location.

3.3.2.3 X-ray DSA Derived Flow Metrics

X-ray DSA images were simultaneously acquired when thermodilution measurements were being made. Like the thermodilution measurements, ten runs with two minute intervals were made for each rat. Each run was composed of 30 x-ray images and one contrast injection. The images were taken at the same point in the cardiac cycle (systole) during respiration apnea. The micro-injector delivered one 100±11 µL bolus of heated contrast agent for each run into the right atrium via the right jugular vein. The same injection catheter (Figure 34a) was used for both x-ray DSA and thermodilution CO measurements.

To measure the cardiac output, a nonparametric deconvolution technique using singular value decomposition (SVD) was used to find relative flow rates. The SVD technique was based on work done by Ostergaard et al. This method was chosen because it is independent of mathematical tissue models in estimating flow rate and thus allows for measurements on a vessel specific basis for pulmonary blood flow (PBF) and relative pulmonary blood volume (PBV), and on a pixel-by-pixel basis for mean transit time (MTT). Briefly, flow rate in a vessel can be estimated by deconvolving the effects of the vasculature from contrast concentration curves. In this case, the curves are pixel value changes of x-ray intensity over time (time density curve, TDC) as the contrast agent moves into and out of a region of interest in a vessel. Typically, an input (arterial
input function, AIF) in the shape of a square impulse function at a proximal vessel location will become a delayed, dispersed curve at a distal vessel location. There are other ways of deconvolving, such as an inverse Fourier transform. However, the SVD technique is the best method. Relative blood volume can be estimated from the area under the time-concentration curve normalized to the area under the AIF curve. The MTT can be calculated by the blood volume divided by the flow rate. For each run, regions of interest (ROI) were taken of the ascending aorta and the pulmonary artery root (AIF). The aortic and AIF concentration time curves were realigned to a common signal maximum, very similar to. This was done to correct for bolus delay associated errors. The ROIs were carefully drawn so that no overlying enhanced blood vessels were included.

Statistics (average, standard deviation, and coefficient of variation) showing the CO measurements for each rat were computed. The x-ray DSA scaling constant \( K_{DSA} \) was then calculated (Equation 20) for each rat by:

\[
K_{DSA} = \frac{\text{Calibrated thermodilution cardiac output}}{\text{X-ray DSA cardiac output}}
\]

Equation 20: DSA calibration factor

so that the relative x-ray DSA CO measurement matched that rat’s specific calibrated thermodilution CO measurement. Statistics (average, standard deviation, and coefficient of variation) showing the interrelationship of the \( K_{DSA} \) between the rats were then calculated. In a contrast-filled vessel, the signal intensity change after logarithmic subtraction is linearly related to the thickness of the vessel. This was experimentally verified in the live rat by plotting the peak DSA signal intensity change in the right pulmonary artery, left ventricle, parenchyma, and aorta versus injection volumes of 50, 100, and 150 µL (Figure 34).
Figure 34: Peak DSA signal intensity in the right pulmonary artery, left ventricle, parenchyma, and aorta plotted versus injection volume. Two DSA runs in the same rat were made for each injection volume. Note the linearity as measured by the $R^2$ of the linear fits of signal intensity change with various contrast concentrations.

Two DSA runs in the same rat were made for each injection volume for a total of 6 runs. Larger injection volumes resulted in higher contrast agent concentrations in the vasculature and thus greater signal intensity change. Linear fits were applied and the $R^2$ values were all $> 0.97$, indicating the linearity between signal intensity and contrast concentration. This agreed well with our hypothesis and allowed for extension of the calibrated aorta flow metric to be applied to the cardio-pulmonary system. So the calibration factor at the aorta can be applied to other vessels in the cardio-pulmonary system. Because the DSA CO was calibrated to the location dependent (DA or AA) thermodilution results, there was a need to measure how independent the DSA calibration factors are between the DA and AA cases. This was done by comparing the percent difference between the product of calibration factors $K_{Thermo} \times K_{DSA}$ within the DA and AA cases. Expressed mathematically, this was done by combining Equation 19
and Equation 20: \( CO_{\text{Fick}} = K_{\text{Thermo}} \times CO_{\text{thermo}} \) and substituting \( CO_{\text{thermo}} = K_{\text{DSA}} \times CO_{\text{DSA}} \) into the former resulted in

\[
CO_{\text{Fick}} = K_{\text{Thermo}} \times K_{\text{DSA}} \times CO_{\text{DSA}}
\]

Equation 21: Calibration factor between DSA and Fick based cardiac output

where

\( CO_x = \) cardiac output measured by Fick, precalibrated thermodilution, or precalibrated DSA

\( K_{\text{Thermo}} \times K_{\text{DSA}} = \) product of calibration factors

The percent difference was calculated by:

\[
% \text{ difference} = \frac{|K_{\text{DA}} - K_{\text{AA}}|}{\frac{K_{\text{DA}} + K_{\text{AA}}}{2}} \times 100
\]

Equation 22: Percent difference

where

\( K_{\text{DA}} = \) average \( K_{\text{Thermo}} \times K_{\text{DSA}} \) for the descending aorta

\( K_{\text{AA}} = \) average \( K_{\text{Thermo}} \times K_{\text{DSA}} \) for the ascending aorta

Simultaneous acquisition of the x-ray DSA and thermodilution measurements was accomplished by the LabVIEW system integration and by the dual use of the contrast agent as the x-ray and temperature marker. At the same time the ten thermodilution measurements were being made, ten x-ray DSA based CO measurements were made for each of the eight rats. The synchronized acquisition resulted in DSA and temperature curves that follow similar time scales and shapes (Figure 36), thus giving confidence to the calibration of DSA to thermodilution. The samples of DSA images (a-d) correspond to the selected points on the continuously acquired thermodilution temperature change between the right atrium and the aorta, and the DSA pixel intensity
change over time (time density curve, TDC) in the aorta (circle in the DSA images). In specific, Figure 36 shows the initial contrast injection with a. right ventricle filling; b. pulmonary venous phase and left ventricle filling with the beginnings of aortic output; c. left ventricular systole with full aortic output, which concluded with d. complete passing of the contrast agent.

The SVD deconvolution method was then applied on the DSA images to derive relative CO flow metrics. The CO values were then used to determine $K_{DSA}$, the scaling factor linking DSA to thermodilution. Because the thermodilution measurement was sensitive to the temperature probe placement and the DSA was calibrated to thermodilution, the DSA calibration factors were also grouped with thermodilution to the DA and AA cases. The average $K_{DSA}$ of the DA rats was $40.48\pm2.63$ with a $6.5\% \ C_v$ and $22.33\pm1.94$ with an $8.7\% \ C_v$ for the AA rats. A measure of independence was performed between the DA and AA specific DSA and thermodilution calibration factors to see if the DSA calibration factor was affected by the location specific thermodilution calibration factor. This was calculated by multiplying $K_{Thermo}$ and $K_{DSA}$ (Equation 21), essentially calibrating the DSA directly to the Fick CO measurement. The results showed there was a $9.41\%$ difference between the average AA and DA product of calibration factors, ($K_{DA}$ and $K_{AA}$) and thus not a significant difference when compared to the $\sim14\%$ variation in the gold standard Fick method. In addition, this also means that a common scaling factor between DSA and Fick can be applied for when thermodilution measurements are not available. This was found to be $18.90\times CO_{DSA} = CO_{Fick}$. Grouping all eight rat pre-calibrated x-ray DSA measurements produced a high CO consistency with a $13.35\% \ C_v$ (average $1.98\pm0.26$ a.u.). In a contrast-filled vessel, the signal intensity change after logarithmic subtraction is linearly related to the thickness of the vessel $^{54,110}$. So the calibration factor at the aorta can be applied to other vessels in the cardio-
pulmonary system. Figure 38 shows the SVD derived mean transit time color map of the same rat in Figure 36. The PBF and PBV were calculated at discrete regions where the correction for attenuation derived from the vessel thickness could be applied (described in further detail in Chapter 3.3.2.4 SVD Flow Metric Vessel Thickness Correction and Experimental Verification).

Figure 35: SVD derived mean transit time (MTT) color map of the same rat in the previous figure. The transit time dynamics were calculated on a pixel-by-pixel basis. This technique provided the ability to quantitatively measure changes on a regional basis—ranging from major vessels (i.e. aorta) to tissue (i.e. lung parenchyma). There was fast transit time in the major vessels (pulmonary artery–arrow) and higher transit time in the more distal locations where the main vessels became capillaries (lung parenchyma–circles).

The blood transit time was calculated on a pixel-by-pixel basis and allowed for measurement of transit time dynamics on a regional basis—ranging from major vessels (i.e. aorta) to tissue (i.e. lung parenchyma). In the MTT map, there was fast transit time in the major vessels (pulmonary artery–arrow, 0.47 sec) and higher transit time in the more distal locations where the main vessels became capillaries. The lung parenchyma
right lower lobe showed a 1.22 second MTT (large circle) and a 1.32 second MTT in the right upper lobe (small circle). The calibrated blood flow metric was calculated at discrete vessel regions. For example, the distal pulmonary artery branchings measured 48.7 ml/min. The PBV and PBF measurements are valid for specific vessels where the correction for attenuation derived from the vessel thickness can be applied. This is described in further detail in the following section.

3.3.2.4 SVD Flow Metric Vessel Thickness Correction and Experimental Verification

The SVD based flow metric calculation deconvolves an arterial input time density curve (TDC) from a distal vessel TDC. The result of the deconvolution is the residue function—a positive, decreasing curve $\leq 1$ that represents the fraction of contrast agent in the distal vessel at a particular time $^\text{80}$. The maximum height of the residue function from the deconvolved TDC is the relative flow rate $^\text{81, 82}$. Given the same arterial input function (AIF) and only amplitude scaling differences in the distal vessel TDCs, the same scaling applies to the deconvolved result. This is because of the associativity of scalar multiplication with convolution—i.e. $(a \times A) \otimes B = A \otimes (a \times B)$, where $a$ is scalar, $A$ and $B$ are the functions to be convolved, and $\otimes$ represents convolution. Logarithmically subtracted x-ray angiography images produce signal intensity values that are linearly related to the cross-sectional contrast thickness of a vessel $^\text{54, 110}$. At the same flow rate, a contrast-filled vessel that is twice as thick has signal intensity values that are twice as high. So in the application of the SVD-based flow calculations on vessels of different diameters with the same flow rate, a scaled deficiency in the calculated flow result will be experienced that is the same scaled difference in vessel thickness. For example, a 2X thick vessel will report a SVD flow result 2X greater than a 1X vessel under the same flow rate. A way to correct for this vessel thickness modulation of the flow calculation is
to scale all vessels to a common vessel diameter. The vessel thicknesses were found was a threshold based method \textsuperscript{111} and used an approach similar to Clough et al. \textsuperscript{110}. Briefly, a minimum intensity projection of the DSA images was filtered and thresholded to extract the contrast-filled tubes, vessels. A distance transform was performed to find the distance of each pixel in the vessel from the nearest vessel boundary, and skeletonization was performed to locate the pixels on the central axes of the vessels \textsuperscript{112, 113}. A line segment was constructed over each skeleton pixel at an angle perpendicular to the central axis, and the distance value at the skeleton pixel was then used to assign thickness values to all pixels along the line segment from one end of the tube to the other. Assuming a cylindrical vessel parallel to the imaging plane and constant attenuation inside the vessel, a radius-to-thickness transform was applied, $2\sqrt{R^2 - x^2}$, where $R$ is the vessel radius and $x$ is the distance of the pixel along the line segment from the skeleton pixel \textsuperscript{110}. Using the same ROIs for the SVD calculations, the average thickness of a common vessel (aorta in the live animal) was found and served to scale the PBF values in the other vessels. A phantom experiment was performed to test this theory of vessel thickness modulating PBF values and the vessel thickness correction. The phantom (Figure 39) was constructed from four lengths of Tygon tubing (Saint-Gobain Performance Plastics, Akron, Ohio).
Figure 36: The purpose of the flow phantom was to verify the SVD based flow calculations between three vessels of scaled diameter. In this schematic, the phantom was constructed from four lengths of Tygon tubing (A, B, C, D). Tube A contained 500µL of x-ray contrast agent and was the input function for the SVD calculations. All other tubes and connectors were water filled. Care was taken to ensure no air bubbles were present in the phantom. B, C, and D represented the distal vessels of interest. They were equal in length (25cm) and the inner diameters were: B-1.14mm, C-2.06mm, and D-2.79mm. Vessel C was what vessels B and D were scaled to using the thickness correction. The inner diameter of vessel C was 1.81X that of B \( (\text{ID}_C = 1.81 \times \text{ID}_B) \) and 0.74X that of D \( (\text{ID}_C = 0.74 \times \text{ID}_D) \). All tubes were in the imaging field of view. A stopcock was used to direct flow from A to B, A to C, or A to D. A syringe pump ran connected at one side of A delivered water at 50.0 ml/min and x-ray images were acquired every 100ms for 30 seconds.
Tube A contained 500µL of x-ray contrast agent (Isovue370) and represented the AIF in the SVD calculations. Care was taken to ensure no air bubbles were present in the phantom. Tubes B, C, and D represented the distal vessels. They were equal in length (25cm) and the inner diameters were: B-1.14mm, C-2.06mm, and D-2.79mm. Vessel C was what vessels B and D were scaled to using the thickness correction. The inner diameter of vessel C was 1.81X that of B (ID$_C$ = 1.81×ID$_B$) and 0.74X that of D (ID$_C$ = 0.74×ID$_D$). All tubes were in the imaging field of view. A stopcock was used to direct flow from A to B, A to C, or A to D. A syringe pump (Harvard Apparatus, Pump 33, Holliston, MA) connected to A ran at 50mL/min and 10 x-ray images were acquired every second for 30 seconds. Four sets of DSA images were acquired for each of the three distal vessels (12 data sets in total). The images were processed as in the live animal study, the SVD-based flow calculation was performed, and the vessel thickness correction was applied. P-values (one-way ANOVA) of the PBF results with and without thickness correction were determined between tube B & C and D & C (tube C was what tubes B and D were scaled to using the thickness correction).

In the flow phantom experiment (Figure 39), tube C was what the small tube B and larger tube D were scaled to for the PBF thickness correction. Representative TDCs of the flow phantom study (Figure 37) showed AIF curves of similar shape.
Figure 37: Representative time density curves (TDCs) of the flow phantom at the AIF and the distal vessels. An external pump was used to deliver the same flow rate through the three tubing sizes. The common tube (Figure 39, A) produced very similar shaped AIF TDCs (tubes B-, C-, D-AIF) in terms of maximum and area under the curve. The distal vessel TDCs (tubes B-, C-, D-Distal Vessel) presented with scaled versions related to the thickness of the tubes. The area under the tube C distal vessel TDC (what the other tube thicknesses were scaled to) was 1.83X larger than tube B and 0.87X smaller than tube D. Similarly, the maximum tube C distal vessel TDC was 1.97X larger than tube B and 0.74X smaller than tube D. Tube C had an inner diameter of 2.06mm and was 1.81X that of B (1.14mm) and 0.74X that of D (2.79mm).
The maximums of the AIF curves were 0.39, 0.36, and 0.32 a.u. (average 0.36±0.04 a.u., $C_v = 9.78\%$) and the areas under the AIF curves were 19.69, 19.20, and 21.00 a.u. (average 19.96±0.93 a.u., $C_v = 4.66\%$) for tubes B, C, and D, respectively. In the distal vessel TDCs, the maximums of the TDC curves were 0.025, 0.049, and 0.065 a.u. and the areas under the TDC curves were 2.98, 5.45, and 6.28 a.u. for tubes B, C, and D, respectively. As the tubing diameter increased, so did the maximum of the TDC and the area under the curves. The SVD based flow calculations across the four data sets for the three tube sizes resulted in PBF values of 0.044±0.006, 0.078±0.003, and 0.093±0.011 a.u. for tubes B, C, and D, respectively. There was statistically significant difference between tubes B & C (p-value < 0.0001) and tubes D & C (p-value < 0.035). Here again, the pre-thickness corrected PBF values increased with increase in tube diameter even though the same pump rate was used. Post vessel thickness correction, the PBF values were 0.079±0.007, 0.078±0.006, and 0.074±0.008 a.u. for tubes B, C, and D, respectively. There was no statistically significant difference after the thickness correction between tubes B & C (p-value > 0.839) and tubes D & C (p-value > 0.471), an indication that matches the reality of the pump being operated at the same rate for all three tubes. In addition, there was no statistically significant difference (p-value > 0.828) before and after the thickness correction for tube C because the scaling was to itself.

The SVD method assumes no contrast agent dispersion. The effect of contrast dispersion can be corrected for by using a finite element vasculature model approach. However, this approach to date is a vessel specific solution rather than a general body solution. Contrast bolus delay effects were corrected by realigning the TDCs and the AIF to a common signal maximum. The extendibility of the SVD method across various time density curves is important in the application of the DSA calibration across a range
of rat sizes and blood flow. Rats of different anterior-posterior dimensions would result in detected x-ray signal intensity changes inversely proportional to the object thickness due to material attenuation. However, signal intensity shifts because of specimen anatomy is suppressed while the contrast filled vasculature is highlighted in DSA because of the logarithmic subtraction,\textsuperscript{36} which mitigates the TDC amplitude dependency on the specimen thickness. However, variations in contrast filled vessel thickness can modulate PBF because of the scalar associativity or multiplication in convolution. So, the vessel thickness modulation of PBF was corrected by scaling the vasculature to a common vessel thickness. In this case, the aorta is the common vessel because this is where the flow measurements were calibrated. The SVD based flow calculations are sensitive to vessel thicknesses because of the associativity of scalar multiplication in convolution. More specifically, vessels/tubing with different diameters delivering the same flow will have a scaled SVD PBF result reflective of the scaling between the vessel thicknesses. So scaling the PBF result by each vessel’s thickness to a common vessel (aorta) can mitigate the vessel thickness PBF modulation. A flow phantom experiment was conducted to verify. The common vessel (Figure 39, tube C) had a inner diameter of 2.06mm and was 1.81X that of a smaller tube (B-1.14mm) and 0.74X that of a larger tube (D-2.79mm). Similarly, the pre-thickness corrected PBF values were 1.77X larger in the common tube (C) than the smaller tube (B), and 0.84X smaller than the larger tube (D) even though the all tubes delivered the same flow rate as set by an external pump. The vessel thickness correction accounted for the small tube (B) PBF deficiency (<1.19 % difference than common vessel PBF) and large tube (D) PBF overestimation (<4.98 % difference than common vessel PBF). This same vessel diameter correction technique was applied to the live animal data for vessel thicknesses that could be measured. A plot of the maximum signal change versus vessel diameter for 50, 100, and 150\(\mu\)L contrast agent injections is below.
Figure 38: Peak DSA signal intensity plotted versus vessel thickness for 50, 100, and 150µL contrast agent injections. Note the linearity of the fits, R^2>0.93.

Like the peak intensity vs. concentration curve in Figure 34, there was a high degree of linearity as measured by the R^2 (> 0.93) of the linear fits. The lung parenchyma was an area where the capillary sizes were smaller than what the x-ray detector could resolve. This resulted in a blush of the region, rather than clearly defined vasculature. So the thickness correction was not applied to these areas and thus PBF values are presented only for specific vessels where the correction for attenuation derived from the vessel thickness can be applied. A projection of the parenchyma could be treated is the superposition of many overlapping capillaries. It is possible to use a volumetric imaging modality like microCT to find the thickness of the parenchyma at each pixel. A volumetric coronal dataset of the segmented lung would be flattened to produce a 2D projection image. This projection image would represent the thickness of the parenchyma at each pixel. Lastly, the projection image would be registered to and applied as a thickness correction for the DSA image sets. Some work has already been done in combining DSA, a projection based imaging method, with CT, a tomographic method. 84
3.4 Small Animal Dosimetry

Efforts to obtain the optimal x-ray spectra for the highest signal-to-noise ratio (SNR) and contrast-to-noise ratio (CNR) have pointed to the use of high-flux x-ray sources with short exposure times (10 ms). On the other hand, the search for better imaging methods should consider radiation damage to the small animal. It has been reported that DNA damage can be caused by a dose as small as 1.2 mGy using 90 kV.

Current methods for small animal dosimetry have several challenges. The traditional method of thermoluminescent dosimetry (TLD) is a labor-intensive and time-consuming process that involves annealing, post-irradiation waiting, and the reading of individual chips. Immediate results can be obtained using an ionization chamber in conjunction with the TG-61 protocol (American Association of Physicists in Medicine). However, inserting the ionization chamber into a live animal is physically unfeasible (the mouse is smaller than the ionization chamber). In live animals, TLDs may be invasively inserted; however, the procedure of inserting and extracting the dosimeter is inconvenient. This is especially true if dose measurements are needed for multiple x-ray techniques. These challenges can be overcome with the use of the metal oxide semiconductor field effect transistor (MOSFET) technology. The advantages of the MOSFET system include: miniature detector size (2.5 mm x 8 mm x 1.3 mm thick), prompt results readout, and multiple dose measurement capability using multiple x-ray techniques with the same detector.

This study involved validating the MOSFET method in comparison with the TLD method as the standard of reference. In the clinic, much work has already been done in this area. In the small animal diagnostic imaging environment, this has yet to be studied.
The scan parameters were 70 kVp, 400 mA and 45 kVp, 400 mA representing the high and lower SNR small animal DSA x-ray techniques (3.1.1 X-ray Beam Optimization). The typical DSA technique used for our small animal imaging consisted of a series of 30 exposures.

### 3.4.1 Mobile MOSFET Wireless Dosimetry System

Dose measurements were made with a mobile MOSFET wireless dosimetry system (Model TN-RD-70-W, Best Medical Canada, Ottawa, Canada). The system included a Bluetooth wireless transceiver (TN-RD-38), a mobile MOSFET reader module (TN-RD-16), and three high-sensitivity MOSFET detectors (TN-1002RD). The MOSFET measured the difference in threshold voltage before and after an x-ray exposure. The voltage difference was proportional to the absorbed dose. Threshold voltages were read immediately after each exposure.

### 3.4.2 MOSFET Calibration

The MOSFET detectors were calibrated at 70 and 45 kVp using two ion chambers–Radcal 6 cc (Model 10x5-6) for the 70-kVp beam and a mammographic ion chamber (Model 10x5-6M-3, Radcal) for the 45-kVp beam–together with a monitor (Model 9015, Radcal, Monrovia, CA). MOSFETs were exposed to approximately 1 Roentgen (R) three times at the two tube potentials. The MOSFETs were placed at 61 cm source-to-dosimeter distance, with the epoxy bulb facing the beam. Calibration factors for each MOSFET were determined by recording detector response in millivolts (mV) and normalizing by absorbed dose (Gy). The conversion of exposure (C kg\(^{-1}\)) to absorbed dose in soft tissue (Gy) was estimated using the f-factor in Hendee’s description, as follows:
\[ D_{\text{tissue}}(\text{Gy}) = f_{\text{tissue}}(\bar{E}) \left( \frac{\text{Gy}}{C \text{ kg}^{-1}} \right) CF_{\text{chamber}} CF_{\text{temp}} CF_{\text{pressure}} X(C \text{ kg}^{-1}) \]

**Equation 23: Exposure to dose conversion.**

where \( D_{\text{tissue}}(\text{Gy}) \) is the dose to soft tissue, \( X(C \text{ kg}^{-1}) \) is the ion chamber reading,

\[ f_{\text{tissue}}(\bar{E}) = \frac{[\mu_{en}]_{\text{tissue}}}{[\mu_{en}]_{\text{air}}} \]

**Equation 24: f-factor**

where \([\mu_{en}]_{\text{tissue}}\) and \([\mu_{en}]_{\text{air}}\) are the mass energy absorption coefficients for the tissue and air, respectively. In Equation 23, the \( CF_{\text{chamber}} \) is the chamber correction factor (\( CF_{\text{chamber}} = 1.012 \) for the 10x5-6 model, and \( CF_{\text{chamber}} = 1.007 \) for the 10x5-6M-3 model, calibrated at the University of Wisconsin Dosimetry Laboratory, Madison, WI). \( CF_{\text{temp}} \) and \( CF_{\text{pressure}} \) are the temperature and pressure correction factors, respectively.

The f-factors as a function of effective energy were fitted to the Boltzmann sigmoidal curve (Prism, version 4.0, GraphPad Software, San Diego, CA), which enabled obtaining f-factors at the appropriate effective energies. The beam quality (HVL) and effective energies for 70 and 45 kVp were obtained by using the data from the Institute of Physics and Engineering in Medicine (IPEM) report 78. The effective energies were estimated to be 40.4 keV for 70 kVp with an f-factor (cGy / R) of 0.9235, and 30.9 keV for 45 kVp with an f-factor of 0.9201.
3.4.3 Mouse Phantom, Dose Measurement, and Statistical Analysis

A tissue equivalent mouse phantom (CIRS, Norfolk, VA) with a diameter of 20.0 mm and a length of 80.0 mm was placed in the x-ray beam path with its long axis perpendicular to the beam (Figure 39).

![Diagram of mouse phantom](image)

**Figure 39:** Schematic of tissue-equivalent mouse phantom. The top drawing is a side view, and the bottom is a top view (looking down). Thus, these two drawings are perpendicular views to each other. The MOSFETs and TLDs were placed on the surface (skin) and interior (body) of the phantom. The phantom was aligned under x-ray guidance to be at the center of the x-ray beam path and with the dosimeters perpendicular to the imaging surface.

Two holes, 5.0 mm in diameter, were drilled in two locations in the interior of the phantom, perpendicular both to its long axis and to the beam direction, to accommodate the MOSFET detectors and TLD chips. One MOSFET detector was placed in each hole. The phantom interior readings (body) were averaged together. The third detector was located on the surface (skin), with the bulb facing the x-ray tube. In separate runs, the MOSFET dosimetry readings were validated by TLD chips (TLD-100, Thermo Scientific, Franklin, MA). The TLDs were placed at the same mouse phantom locations as where
the MOSFET measurements were made. Three MOSFET detectors measured the dose for five DSA runs (30 exposures per run) using the settings described earlier. At the same three locations, six TLD runs were made at each tube potential. The average dose and standard deviation ($1\sigma$) were calculated for both methods. Bland-Altman analysis was used to compare agreement between the two methods (MOSFET versus TLD). All of the dose values were used to produce a Bland-Altman plot. The plot is a calculation of the average of the two values (MOSFET and TLD for each location) and the difference between the two measurements (Prism, version 4.0).

### 3.4.4 Results

The MOSFETs were validated to TLDs in the small animal imaging environment. Figure 40 shows the dose imparted at the body and skin locations in the mouse phantom.

![Figure 40: Dose responses at the phantom surface (skin) and interior (body) measured using MOSFETS and TLDs at 70 and 45 kVp. Both dosimeters were exposed to the same x-ray techniques (30 exposures, 400mA, 10ms). As expected, the dose was higher at the phantom surface and at the higher tube potential.](image-url)
At 70 kVp, the MOSFET reported 2.04±0.01 and 2.21±0.05 cGy for the body and skin locations, respectively; the TLDs reported 1.99±0.06 and 2.43±0.17 cGy at the same respective locations. The MOSFET reported 0.69±0.06 and 0.84±0.04 cGy for the body and skin locations at 45 kVp; the TLDs reported 0.71±0.02 and 0.90±0.06 cGy for those same locations. The energy dependence of TLDs was determined as 0.036±0.002 R/mV and 0.030±0.002 R/mV for 45 kVp and 70 kVp respectively; for MOSFETs 14.4±0.4 mR/nC and 15.8±0.9 mR/nC for 45 kVp and 70 kVp respectively. Bland-Altman analysis showed a bias of 0.0625. In 95% of doses, the difference lies between -0.16 and +0.29. The standard uncertainty (spread of individual measurements from the average) was 0.4-8.3% for MOSFET and 2.8-7.0% for TLD.

Bland-Altman analysis provided a simple method of plotting the comparison of two methods. The plot in Figure 41 shows the average of the two measurements (MOSFET and TLD) on the x-axis and the actual difference between the two measurements on the y-axis. The bias of nearly zero (0.0625) indicates the relatively comparable nature of the two methods.
Figure 41: Graph shows Bland-Altman plot for difference between measurements versus averaged measurements. Dotted line shows bias (Y=0.0625).

The focus of this work is for application in low dose small animal diagnostic imaging where the standard uncertainty is expected to be broader (<8%) than for radiation therapy (1-2%) due to photon statistics. The tissue equivalent mouse phantom was unique in that the detector placement holes were drilled perpendicular to the long-axis of the phantom to avoid any attenuation from the copper MOSFET cables overlapping other dosimeters. Both MOSFET and TLD dosimeters showed the energy dependence and this has been incorporated in the calibration factors for the respective tube voltages.

As shown in Figure 40, doses were consistent with material attenuation, with the higher doses at the surface (skin) and lower doses in the phantom (body). Based on the MOSFET results between the 70 and 45 kVp scans, the dose was approximately 200% higher in the body and 160% higher at the surface. Likewise, higher tube potential resulted in higher dose. At the same tube potential (70 kVp), the dose was 8% higher on the skin than in the body. At the lower energy (45 kVp), the dose was 22% higher on the
surface than in the phantom interior. More of the 70 kVp spectra passed through the phantom than was absorbed. The lower energy imparted relatively more dose to the phantom interior than at the higher energy. This is because of the low energy x-ray absorption in a thin specimen (20 mm diameter).

The MOSFET dosimeter reported immediate results and the same detector can support multiple exposures with different x-ray techniques (a significant improvement to the hours needed for TLD preparation and reading). MOSFET technology has proved to be a fast, reliable small animal dosimetry method, and is a good system for dose monitoring for serial and gene expression studies.

3.5 System Integration

This section describes the computer infrastructure (hardware and software) that allowed for physiological event driven imaging (the biological pulse sequence), the software for digital subtraction, and the image archival system.

3.5.1 Biological Pulse Sequence

The ability of our unique system to synchronize small animal breathing, monitoring, and contrast injection with exposures and camera read allows for the concept of a biological pulse sequence. This event driven sequence is analogous to the encoding sequences used in MRI where a line of K-space is acquired each time a RF pulse is played out. Here instead, a 2D array is acquired for each radiographic exposure pulse. A typical sequence (Figure 42) to measure pulmonary blood flow involves subtraction images acquired at the same point in the breathing cycle and at end systole in the cardiac cycle with a series of injections ranging from 15 to 20 microliters in the mouse and 50 to 2000 microliters in the rat.
An example of these time series DSA images for a Fischer 344 rat is seen in Figure 43. A contrast injection was done through the right jugular vein. The contrast moves from the injection site into the right side of the heart (a), then into the pulmonary arteries (b), and into the lung parenchyma (c). The contrast then returns to the heart and is pumped out by the left ventricle to the rest of the body through the aorta (d, e). More peripherally, the carotids and renal arteries are seen (f). Note how the lungs and heart are in the same position in every image because images were acquired at the same point in the cardiac cycle with suspended respiration so subtraction artifacts are not experienced.
Figure 43: Time Series live rat DSA following the biological pulse sequence described in Figure 42.
Other variations of the biological sequence are being explored. For example, imaging at full and at end respiration to study lung volume modulated effects of pulmonary blood flow and cardiac wall motion studies using sequential injections at end expiration and at multiple points in the cardiac cycle. A way to expand the BPS even further is the use of a field programmable gate array (FPGA). This is described more in the 6.3 Biological Pulse Sequence Development, Revisited section. Further detail of the BPS code can be found in the Appendix.

3.5.2 Digital Subtraction Graphical User Interface

A custom MATLAB graphical user interface (GUI) has been developed to perform the digital subtraction and prepare the images for archival (see next section). Additional MATLAB scripts are used to obtain quantitative measurements from the processed data. Descriptions of the software scripts are below. Further detail of the code can be found in the Appendix.

3.5.2.1 Logarithmic Subtraction

The original pre-contrast time series images are averaged to create a mask from which post-contrast time series images are subtracted from, leaving only the contrast enhanced blood vessels. The subtractions are done logarithmically as in $^{54,126}$ so that only the amount of iodine contrast, not the specimen thickness, modulates the signal (Equation 6). An example can be seen in Figure 43.

3.5.2.2 Paradox Subtraction

Another variation of the digital subtraction is to subtract between images $^{65}$. This differential subtraction allows you to follow the bolus of contrast as it moves from the injection site through the body (Figure 44). Dark areas represent where the contrast agent
is present in the current image and areas that are bright represent where the contrast was in the previous frame. This virtual bolus chase can also be used to find ventricular motion\textsuperscript{127}.
Figure 44: Time series live rat DSA using Paradox Subtraction.
3.5.3 Image Archival System

The raw and digitally subtracted images from the DSA GUI above, monitored biological and system signals, and any standard information describing the experiment ranging from amount of contrast injectate to the type of small animal are archived to an online database for post processing, data display, storage, and Internet distribution. A number of data handling steps are performed automatically in the MATLAB GUI to prepare the information for archival. Steps include meta-data organization and other protocols to provide proper handshaking of the data with the CIVM archival system. The effect of this archival system is a data secure repository that collaborators and peers can share image and derived data with searchable tags much like a “Google” search engine. Further detail of the code can be found in the Appendix.

4. Application of DSA System to a Drug Model of Hypertension for Visualization and Blood Flow Measurement on a Regional Basis

The small animal optimized x-ray DSA system was used for studying the changes in the cardio-pulmonary blood flow before and after a vasoconstrictor drug administration\textsuperscript{128}. Phenylephrine was used to perturb the rat cardio-pulmonary vasculature and blood flow as in other rodent studies\textsuperscript{129-133}. This vasoconstrictor was chosen because it lacks chronotropic and inotropic actions on the heart\textsuperscript{132, 134}. Thus, cardiac irregularities are rarely experienced and the perturbations in blood flow are more vascular tonicity than heart rate related. Imaging based measurements were performed at high levels of spatial resolution (100 µm x 100 µm) that included vasculature within the lung and at temporal sampling rates capable of detecting anatomical and
physiological changes in real-time before, during, and after drug administration (the drug administration resulted in rapid anatomical and physiological changes\textsuperscript{134}).

4.1.1 Animal Setup:

All animal studies were conducted with the approval of the Duke Institutional Animal Care and Use Committee. Three catheters were placed in each of the 11 male rats (235±20g, Fischer-344) for x-ray contrast agent injections (right jugular vein at the root of the superior vena cava, PE-50, 22.2 cm length), blood pressure measurements (femoral artery), and drug infusion (tail vein). The x-ray contrast injection system was the same one described in 3.2 High Precision Micro-contrast Power Injector and used in previous studies\textsuperscript{11, 36, 42, 83, 84, 131, 135}. Over the study duration, blood pressure measurements were made (Direct blood pressure kit, DBP1001, Kent Scientific Corporation, Torrington, CT) at the same time points as the DSA image acquisition (described in more detail below). The blood pressure measurements were normalized to pre-drug administration states across the 11 rats. The animals were anesthetized on 1-3% Isoflurane, ventilated (60 breaths/min, 2 cc tidal volume), kept warm with a heat lamp controlled via feedback from a rectal thermocouple (37±0.1°C), and monitored (ECG, blood pressure, etc.), as in previous studies\textsuperscript{36, 42, 83, 84, 135}.

4.1.2 Imaging Protocol:

Each optimized small animal DSA\textsuperscript{36,114} data set was composed of 30 x-ray images and one contrast injection. The images were taken at the same point in the cardiac cycle (systole) during respiration apnea. The micro-injector delivered one bolus (100±11 µL) of contrast agent (Isovue 370, 370mg iodine/mL, Bracco Diagnostics, Princeton, NJ) into the right atrium via the right jugular vein for each DSA data set. DSA data sets were acquired at various time points during vasoconstrictor administration (phenylephrine...
hydrochloride 1%, 10 mg/mL, American Regent Laboratories, Inc., Shirley, NY). The following is a timeline for image acquisition and drug infusion (Figure 45).

![Timeline of events showing the relationship between the DSA imaging and the phenylephrine drug administration.](image)

**Figure 45:** Timeline of events showing the relationship between the DSA imaging and the phenylephrine drug administration. The drug was infused for 2 minutes during which DSA image sets were acquired every 30 seconds, up to 4 min after the start of the first (pre-drug) DSA data set. The remaining image sets were acquired at the 5, 8, and 22 minute time points. A total of 12 DSA data sets (30 images per set) were acquired for each of the 11 rats following this timeline. The pre-drug, post-drug, and recovery time points correspond to the DSA and blood flow maps in the figures below and in the text.

First, a pre-drug DSA scan was performed. Then the vasoconstrictor was infused (7.5 mL/hr) for 2 minutes. At the same time, DSA scans were performed every 30 seconds and continued until 2 minutes after the conclusion of the drug infusion (4 minutes after the start of the drug administration). Finally, DSA scans were performed at 5 (drug recovery), 8, and 22 minutes after the start of the drug infusion. In total, 12 DSA data sets (30 images per set) for each rat were acquired.

### 4.1.3 Blood Flow Quantitation:

To measure the cardio-pulmonary blood flow and related metrics (mean transit time and blood volume), a nonparametric deconvolution technique using singular value
decomposition (SVD) was implemented on the DSA images (3.3.2.3 X-ray DSA Derived Flow Metrics). Contrast bolus delay effects were corrected by realigning the distal TDCs and the AIF to a common signal maximum. The relative measurements produced by the SVD calculations were corrected for vessel thickness modulation of PBF values over specific vessels where the correction for attenuation derived from the vessel thickness could be applied and then the result calibrated to absolute metrics (3.3.2.4 SVD Flow Metric Vessel Thickness Correction and Experimental Verification). In addition, the aorta inner diameter was measured as part of the vessel thickness correction for all the rats and time points. The final products for each DSA data set were calibrated PBF (mL/min) and relative PBV values at discrete regions, and color maps of the mean transit time on a 100 µm x 100 µm, pixel-by-pixel basis. Regions of interest (ROI) were taken of the bilateral pulmonary arteries and aorta for the PBF and PBV measurements. For the transit time measurements, ROIs were taken of the bilateral pulmonary arteries, aorta, left ventricle, and four lung parenchymal areas (upper, apical and lower, basal lobes for each lung). For each region of interest, the average value across the 11 rats at each of the 12 time points was plotted over the 22-minute study duration. To measure the blood flow metric intervariability between the rats at each time point, the coefficient of variation (Equation 25) was calculated as:

\[
\text{coefficient of variation } (C_v) = \frac{\sigma \text{ (standard deviation)}}{\mu \text{ (mean of each ROI value)}}
\]

**Equation 25: Coefficient of variation**

In addition, p-values (one-way ANOVA) were determined by comparing between the 11 rats over all time points for each ROI.
4.1.4 Results:

Figure 46 shows DSA images for one rat that are representative of the study group. The images shown were acquired at pre-drug (0 min), immediately post-drug administration (2 min), and recovery (5 min) from the phenylephrine infusion. The time series images for each DSA run (3 columns) were acquired at the same time point in the cardiac cycle with a steady heart rate of 370 ± 40 beats per minute between the DSA runs. One 100 ± 11 μL contrast injection was made for each DSA run. The contrast movement can be followed through time from the injection site in the right heart (162 ms after contrast agent injection), to the lungs, and finally to the systemic system (2268 ms). The phenylephrine infusion markedly altered the blood flow and the vessel anatomy. This is clearly seen after the drug has been administered for 2 minutes (post-drug)—the pulmonary arteries (black arrows), left ventricle (gray arrow), and aorta (white arrow) are distended compared to the pre-drug and the recovery time points. In addition, almost a full second elapses before the contrast moves into the same phase after the drug injection (2268 ms) when compared to pre-drug and recovery (1296 ms).
Figure 46: The DSA images shown here are of the same rat and are representative of the study group. The columns show DSA runs acquired at pre-drug (0 min), immediately post-drug administration (2 min), and recovery (5 min) from the phenylephrine infusion. The images were acquired at the same point in the cardiac cycle with a steady heart rate (370 ± 40 BPM) between the three DSA runs. The y-axis time scale represents the time from injection of the contrast agent (from the 2\textsuperscript{nd} to the 15\textsuperscript{th} heart beat) for each DSA run. The vasoconstrictor induced peripheral blood vessel constriction that resulted in an increase in central blood volume and thus distention of the major vessels. This is clearly seen after the drug has been infused for 2 minutes (post-drug column)–the pulmonary arteries (black arrows), left ventricle (gray arrow), and aorta (white arrow) are distended compared to the pre-drug and the recovery time points. In addition, almost a full second elapses before the contrast moves into the same phase after the drug injection (2268 ms) when compared to pre-drug and recovery (1296 ms). The cardio-pulmonary system returns to a pre-drug administration state soon after the drug infusion was stopped. This can be seen in the likeness of the DSA images (1\textsuperscript{st} and 3\textsuperscript{rd} columns) and in the quantitative metrics (SVD transit time maps, mean arterial pressure, and aorta inner diameter) described in the following figures and in text.
The pre-drug and recovery images are very similar qualitatively, as seen in this figure, and quantitatively (blood pressure, aorta inner diameter, and MTT), as reported by the blood flow quantification results. Color maps of blood transit time for the same rat as the previous figure are shown in Figure 47, with measurements taken at the same time points--pre-drug (0 min), immediately post-drug administration (2 min), and recovery (5 min). The PBF (mL/min) and PBV (a.u.) values at specific regions (aorta and bilateral pulmonary arteries) were calculated as in previous work\textsuperscript{135} (3.3.2.3 X-ray DSA Derived Flow Metrics).

![Figure 47: SVD derived blood transit time color maps of the same rat at the same time points in the previous figure. Like the x-ray DSA images in Figure 46, the pulmonary arteries (unfilled arrow) and aorta (solid arrow) are distended after drug administration. The MTT substantially increased throughout the cardio-pulmonary system (quantitatively presented in Figure 48). The MTT increase, especially in the lung parenchyma (circles), is the result of the phenylephrine constricting the capillaries. Note how parenchymal perfusion was altered because the pulmonary artery transit time increased (box) after the drug administration because the vasoconstrictor closed off the distal pulmonary artery branching. In recovery, some of the pulmonary artery branching have reperfused.](image-url)
Like the x-ray DSA images, the pulmonary arteries (unfilled arrow), left ventricle, and aorta (solid arrow) are distended after drug administration. In addition to the anatomical changes, the mean transit time (MTT) substantially increased. The retardation in blood flow, especially in the lung parenchyma (circles), is the result of the vasoconstrictor causing arteriole constriction. The lung capillary density varies between the apical/basal and the proximal/dial hilum areas and so the effect of the vasoconstrictor is not uniform. Because the transit time dynamics were calculated on a pixel-by-pixel basis, this technique provided the ability to quantitatively measure the non-uniform changes for both major vessels (i.e. aorta) and tissue regions (i.e. lung parenchyma). To fully harness this capability, regions of interest highlighting vessels and tissue were chosen and plots of the transit time changes during the course of the experiment are show in Figure 48 for 11 rats over the 22-minute study duration, from pre-drug to drug administration to recovery. The ROIs were the pulmonary arteries, left ventricle, aorta, and four areas of lung parenchyma (upper/apical and lower/basal lobe areas for each side of the lung). In addition, Figure 48 graphically shows the average SVD derived relative blood volume and blood flow at specific vessels (bilateral pulmonary arteries and aorta) for where the correction for attenuation derived from the vessel thickness could be applied.
Figure 48
Figure 48: These graphs show the average blood pressure normalized to pre-drug (time at 0 min) and average SVD derived blood volume, flow, and mean transit time metrics (left and right body side) for 11 rats over the study duration, from pre-drug to drug administration to recovery. The ROIs for the SVD calculations are of the major vessels, heart, and lung parenchyma specific to each SVD derived metric of the 11 rats at each imaging time point. The drug was infused for 2 minutes after pre-drug. DSA runs were gathered every 30 seconds for 4 minutes after pre-drug, and then at time points 5, 8, and 22 minutes after pre-drug. The drug had a dramatic effect on the blood pressure, aorta inner diameter, and SVD derived metrics. Once the drug infusion was stopped at 2 minutes, the perturbed physiology quickly reverted to original values. By the 5 min time point (recovery), 3 minutes since post drug infusion, most values returned to pre-drug. For example, the aorta inner diameter steadily increased (from 2.28 mm to 2.64 mm at max) and followed similar trend as the blood pressure changes with the introduction of the vasoconstrictor, and then steadily declined post drug administration back to pre-drug values at recovery. The significant increase in MTT (up to 27.5% in the right lower lung lobe) and decrease in flow (up to 48.9% in the left pulmonary artery) was expected because the drug constricts tissue capillaries and thus increases the vascular resistance, which impedes flow. The aorta inner diameter and blood pressure graphs show a ±1 standard deviation. Standard deviations are not shown in the other plots because the graphs would become too cluttered. Instead, coefficient of variation values are presented in the text. In addition, p-values (one way ANOVA) were determined by comparing between the 11 rats over all time points for each ROI. P-values > 0.05, showing statistically no significant difference are highlighted with an * next to the ROI label. (LV-left ventricle; R. PA-right pulmonary artery; L. PA-left
pulmonary artery; RU, LU-lung parenchyma right, left upper/apical lobe; RL, LL-lung parenchyma right, left lower/basal lobe).
The maximum relative PBV decrease was found in the left pulmonary artery (8.7%). The PBF dramatically changed between pre-drug and the 2 min time point. The left and right pulmonary arteries showed the most marked change with a 48.9% (117.3 to 59.9 mL/min) and 34.25% (105.5 to 69.3 mL/min) drop in flow, respectively. The left ventricle showed a 20.6% reduction in flow from 33.7 to 28.8 mL/min. The drug also had pronounced effects on increasing the MTT between pre-drug and immediately post-drug administration in the left ventricle (22.4%), aorta (12.6%), and lung parenchyma (RU, 36.1%; LU, 34.3%; RL, 32.7%; LL, 36.2%). The significant MTT increase in the lung parenchyma was expected because phenylephrine constricts tissue capillaries. This increased the vascular resistance and thus impedes flow.

The lowest coefficient of variation across all regions of interest and all time points for 11 rats was 7.37% (right pulmonary artery-4min), 10.56% (right pulmonary artery-1min), and 4.93% (lung lower left lobe-3min) for PBV, PBF, and MTT, respectively. More specifically, the average standard deviation at those time points and locations across the 11 rats were 0.09a.u., 8.67mL/min, and 0.04sec for PBV, PBF, and MTT, respectively. Assuming that the standard errors (σ) of each blood flow metric are equal, the smallest detectable difference with a 90% confidence interval is $1.64 \times \sigma \sqrt{2}$

So, a change in blood flow metrics would need to be greater than 0.21a.u. (PBV), 20.11mL/min (PBF), and 0.09sec (MTT) for this technique to be sensitive to a minimal difference in hemodynamics.

The one-way ANOVA results of the PBV values showed no statistically significant difference (p-value > 0.05) over the study duration (12 time points) for 11 rats in all of the regions: p-value = 0.358-aorta, 0.748-R. PA, 0.240-L. PA. This too was the case for all of the PBF values, all ROIs showed no statistically significant difference across the study duration for all rats (p-value = 0.331-aorta, 0.089-R. PA, 0.469-L. PA).
The MTT results showed no statistically significant difference in the ROIs of the left and right pulmonary arteries, and left and right lung upper lobes (p-value = 0.080-R. PA, 0.494-L. PA, 0.157-lung RU, 0.216-lung LU). Statistical significance was found for the aorta, LV, and bilateral lung lower lobes (p-value = 0.004-aorta, 0.001-LV, 0.032-lung RL, 0.016-lung LL).

Another imaging derived measurement was the inner diameter of the aorta. This measurement was made possible because of the inherent vessel enhancement of DSA and the rapid, synchronized physiological driven image acquisition. In addition, a direct physiological measurement was made of the blood pressure. The results presented describe the average blood pressure normalized to pre-drug (time at 0 min). The 2-minute drug infusion had a dramatic effect on the blood pressure and aorta caliber just like the SVD derived metrics. Once the drug infusion finished, the perturbed physiology quickly reverted to original values. By the 5 min time point (recovery), 3 minutes after post drug infusion, most values returned to pre-drug conditions. Note how the blood pressure steadily rose and the aorta inner diameter followed the same trend with the introduction of the vasoconstrictor, and then steadily declined post-drug administration back to pre-drug values during recovery. There was a 103.95% increase in the average normalized blood pressure between baseline (0 min) and after drug injection (2 min). The aorta inner diameter also showed an increase over baseline, from an average inner diameter of 2.28 mm to 2.64 mm, a 16.06% increase.

4.1.5 Discussion:

DSA images and blood pressure measurements were acquired at various time points before, during, and after a vasoconstrictor administration. From the DSA images the aorta caliber was measured and blood flow dynamic maps of vessels and tissue space and specific vessels were derived using a non-parametric deconvolution technique. This
technique provided calibrated mL/min blood flow and relative blood volume
measurements at specific blood vessels and transit time dynamics on a regional, pixel-
by-pixel level. To observe the drug effects in specific areas, multiple regions of interest in
the cardio-pulmonary system were chosen. These ROIs encompassed major vessels and
parenchymal space. Blood flow metrics in those ROIs across 11 rats were plotted over
the duration of the experiment. In the section below, we discuss the qualitative and
quantitative changes in the cardio-pulmonary vasculature and blood flow due to the
phenylephrine administration.

The DSA images were acquired during respiration apnea and at the same point
during every cardiac cycle. Thus, there were no subtraction artifacts (blurring or
shadowing) in the images and the physiological state could be matched and compared
between the DSA runs. Post-drug infusion DSA images showed dramatic distention in
the major vessels and retardation in blood flow. The anatomical and physiological
changes returned to pre-drug states ~3 minutes after the drug infusion was stopped.

Phenylephrine acts primarily on the arterioles. The vasoconstrictor induced
peripheral blood vessel constriction, which caused an increase in peripheral vascular
resistance (PVR) and thus an increase in central blood volume (CBV). To preserve
conservation of blood volume, this increase in CBV caused distention of the major
vessels. The increase in CBV and the greater PVR caused the mean arterial pressure rise
to ~2X and the average aorta inner diameter to increase ~1.2X that of pre-drug versus
recovery. In addition, the blood flow transit time, especially in the parenchymal tissue,
became dramatically retarded by almost a full second. This is significant given the
context of the rat’s ~400BPM heart rate.

A deconvolution technique was performed on the x-ray DSA images to produce
maps of blood transit time, and discrete blood flow and relative blood volume metrics
at specific vessels. This technique provided the ability to quantitatively measure changes
in blood flow metrics on a pixel-by-pixel regional basis for the transit time maps and vessel-specific measurements for PBF and PBV, a substantial improvement over previous methods that could only provide regional information at the major vessel sectional, or organ level. In addition, only a few studies were performed in the rat. The other studies were performed in canines, lambs, and humans. To highlight local changes in blood transit time across each 22-minute experiment, ROIs were drawn in major vessels and parenchymal spaces. ROIs of the major vessels (bilateral pulmonary arteries and aorta) were used for the PBF and PBV measurements. In Figure 47, the vasoconstrictor significantly increased the blood transit time (MTT > 25% increase in the parenchymal ROIs from pre-drug) between baseline (0min) and post-drug (2min). The MTT increase, especially in the lung parenchyma (circles), is the result of the phenylephrine constricting the arterioles. The pulmonary arteries (unfilled arrow) and aorta (solid arrow) are distended after drug administration. Note how parenchymal perfusion was altered because the pulmonary artery transit time increased (box) after the drug administration because the vasoconstrictor closed off the distal pulmonary artery branching. In recovery, some of the pulmonary artery branching have reperfused.

P-values were found (one-way ANOVA) for each blood flow metric (3 total–PBV, PBF, MTT) between the 11 rats over all time points (12 total) for each ROI (8 total for MTT, and 3 each for PBV and PBF). Overall, minimal statistical significant differences were found, indicating that the blood flow metric changes before, during, and after the drug administration were primarily due to the vasoconstrictor properties of phenylephrine rather than imaging or rat variability. Statistical significance was found only for the aorta, LV, and bilateral lung lower lobes for MTT. The ROIs were drawn with attention to minimizing vessel/parenchymal overlap in light of the superposition nature of planar imaging. The superposition/overlap of vessels and parenchyma may
explain the MTT curve inflections between the left and right pulmonary arteries and the statistically significant changes between rats at some ROIs. Nevertheless, the same approach could be extended to tomographic DSA to mitigate superposition effects. The DSA and SVD based blood flow calculations provided a solution for providing regional measurements in the live animal. In addition, the measurements were made on a temporal scale (sampling every ~30 seconds with imaging at every heart beat during each sample) where rapid, acute changes in anatomy and physiology could be acquired. In this case, the physiology reverts back to original states in minutes and thus required a measurement solution, which this technique fulfills, with rapid temporal sampling on the scale of seconds.

4.1.6 Application of DSA System on a Drug Model of Hypertension Summary:

In this work, phenylephrine was infused to perturb the cardio-pulmonary vasculature and blood flow metrics. X-ray DSA images were acquired before, during, and after vasoconstrictor administration, with a total of 12 DSA sets per rat (11 animals total) over 22-minutes. The drug caused major vessel distention, blood flow retardation, increase in blood pressure, and increase in aorta caliber. After the drug infusion finished, the anatomical and physiological changes returned to pre-drug values. SVD deconvolution based flow metrics calculated from the DSA images generated maps of mean transit time changes on a pixel-by-pixel (100 µm x 100 µm) basis, and discrete measurements of blood flow and relative blood volume at specific vessels where the correction for attenuation derived from the vessel thickness can be applied. This system can detect changes in hemodynamics on the order of 0.21a.u. in relative blood volume, 20.11mL/min in blood flow, and 0.09sec in transit time. The ability to measure absolute blood flow, relative blood volume, and transit time dynamics on this level of regional
specificity and at these resolutions, and to do so rapidly (acquiring data at every heart beat) while the animal is alive suggests that this imaging technique can provide an exciting solution for in-vivo functional phenotyping. Applications include blood flow modulation by drugs (e.g. nitroprusside), disease (e.g. lung perfusion induced by hypoxia), and/or genetic models (e.g. spontaneous hypertension). This technique could also be extended to hepatic, renal, tumor blood flow measurements and vessel visualization \(^{42,83}\).
5. Conclusions

Small animal functional imaging provides a way to measure physiologic changes minimally-invasively while the animal is alive, thereby allowing for multiple measurements in the same animal with little physiologic perturbation. As such, there has been development of many small animal imaging devices (microCT, microPET, ultrasound, MRI microscopy, optical imaging, etc.), but they are not optimized for the small animal in terms of minimally invasiveness, sampling speed, spatial resolution, vascular visualization, and blood flow measurements in absolute units. The focus of this work was the development of a small animal x-ray imaging system capable of minimally invasive real-time, high resolution vascular visualization, and cardio-pulmonary blood flow measurements in the live animal.

This small animal DSA system can perform: 1) minimally invasive in vivo blood flow measurements, 2) multiple measurements in the same animal in a rapid succession (every 30 seconds—a substantial improvement over singular measurements that require minutes to acquire by the Fick method), 3) very high resolution (up to 46 micron) vascular visualization, 4) quantitative blood flow measurements in absolute metrics (mL/min instead of arbitrary units or velocity) for where the correction for attenuation derived from the vessel thickness can be applied and relative blood volume dynamics from discrete ROIs, and 5) relative mean transit time dynamics on a pixel-by-pixel basis (100 μm x 100 μm). The end results are 1) anatomical vessel time course images showing the contrast agent flowing through the vasculature, 2) blood flow information of the live rat cardio-pulmonary system in absolute units and relative blood volume information at discrete ROIs of enhanced blood vessels, and 3) colormaps of relative transit time dynamics. This approach is a planar imaging method and thus the superposition of
enhanced structures can result in over estimation of relative blood volume. The images for the calibration and drug study were acquired at detector pixel resolutions of 100 µm², so blood vessels smaller than that size could not be resolved. Vessels that were smaller than 100 µm presented as a blush in the DSA images. This could be seen in the parenchymal space. In those areas, the SVD vessel thickness correction could not be applied because the vessel sizes are unknown. Thus only vessels that could be resolved were corrected for thickness and then calibrated to absolute flow metrics. The vessel thickness correction assumes a cylindrical vessel that is parallel to the imaging plane.

What makes this DSA system unique is that it has been optimized for and designed around the small animal in terms of: selecting the x-ray technique for the highest SNR, high acquisition speed, high spatial resolution, high image contrast by nature of DSA and the repeatable, real-time physiological driven image acquisition, minimally invasive contrast injection, small animal support and monitoring, and quantitative blood flow analysis. To date, there is no other work that integrates these elements into an operational platform designed explicitly for small animals promise an exciting solution to obtaining real time anatomical and quantitative functional information in the live rodent.
6. Future Work

6.1 Second Generation X-ray System

The design and implementation of this work has spanned multiple disciplines and the results have allowed further instrumentation development. The x-ray system in this work has been expanded through the construction of a new 2nd generation system. The optimized x-ray techniques, power micro-contrast injector, small animal support, biological pulse sequence, and image-based blood flow measurements from the 1st generation system were directly applied to the new system. One of the significant improvements is that there are two pairs of x-ray sources and detectors (Figure 49).

![Figure 49: Second generation x-ray system](image)

The dual chain allows for faster CT sampling and multi-angle DSA imaging. An advantage of CT over DSA is that CT results are three-dimensional image volumes. This
provides depth information and eliminates the superposition effects experienced in projection imaging. DSA has the advantage in sampling rate—an entire dataset showing the cardio-pulmonary vasculature is gathered in < 8 seconds. In addition, DSA provides vascular visualization and blood flow information. In previous work, microCT and microDSA were combined to harness the qualities of each imaging specialty. The results showed vascular visualization and blood flow information through a depth of the cardio-pulmonary system. The sampling strategy used involved capturing multiple DSA acquisitions with limited projection angles (up to 80°), a tomosynthetic approach. An important element in the sampling strategy was limiting the total amount of injected contrast agent to be < 10% of the total blood volume to limit physiological impact. With the new 2nd generation system, twice as many projection angles can be acquired in the same amount of time and could produce a truly tomographic reconstruction. Another option is to reduce the volume of injected contrast agent by half when operating at similar image quality as in the tomosynthetic approach. In addition, dual energy subtraction imaging can be performed on the new system. Here, the specimen is imaged at “high” and “low” x-ray energies that best straddle the attenuation characteristics of the contrast agent or tissue of interest. Using the natural x-ray attenuation subtleties between different tissues, the subtracted images can produce high contrast soft tissue images.

6.2 Additional Animal Model

This work has been primarily for quantitative blood flow imaging in the rat. However, many key systems and technologies can be applied and are currently available for mouse blood flow imaging. For example, the micro-injector can deliver contrast agent in small volumes (down to 6 µL) suitable for the mouse environment. In addition, the x-ray imaging techniques, blood flow analysis software, and animal support system are all
“mouse-ready.” This small animal DSA system can also be applied to other physiological systems. Calibration of the relative blood flow metrics to absolute units will need to be done for the mouse. A future project could be quantitative blood flow measurements in the mouse renal system. Integrated control of blood flow is critical to regulating virtually all of the functions of the kidney. For example, alteration of blood flow in the renal cortex affects glomerular filtration rate (GFR), whereas control of blood flow in the medulla impacts fluid and solute reabsorption. Several hormone systems are involved in coordinating these hemodynamic functions and the impact of these systems may vary in different regions of the kidney. In this regard, angiotensin II is a potent vasoconstritor acting to increase glomerular hydrostatic pressure while reducing medullary blood flow. In the CRE-Agtra1aflox/flox knockout mouse, the major vasoconstrictor receptor for angiotensin II is deleted from the vascular smooth muscle cells. The knockout mouse is unresponsive to the vasoconstrictor and thus presents with abnormal GFRs. This study could help to define the molecular control of renal hemodynamics by angiotensin II.

**6.3 Biological Pulse Sequence Development, Revisited**

The current hardware/software of the biological pulse sequence supports up to 8 channels and requires external Boolean gates and a myriad of logic cables connecting between the x-ray system, physiologic inputs, computer sequencer, and external Boolean gates. Switching between BPS involves rewiring all of the logic cables between all of the components. This is tedious and limits to kinds of sequences to what can be supported by only 8 channels and a number of external Boolean gates. A solution is the field programmable gate array (FPGA). The FPGA allows reprogrammable logic programming on the computer hardware level and a larger number of supported signals. The National Instruments FPGA, PCI-7811R is currently being tested. This device has 96 channels, 12
times more than what our current system supports, and can be programmed such that all the external logic boxes are eliminated and the number of logic cables can be simplified to just a handful. As of now, a FPGA program has been written that mimics that same BPS as our current cable and Boolean gate assemblies for cardiac gated imaging during respiration apnea like that seen in Figure 42 and Figure 43. This FPGA will allow for easier and more robust switching (simply opening a new FPGA program) between biological pulse sequences while providing for more opportunity to create new sequences.

6.4 Image Archival System, Revisited

The current data archive hierarchy allows raw and processed images, physiologic data, and derived measurements to be stored. To extend the scope of data archival, the biological pulse sequence can be stored for reference and for use in future experiments. Linking between images, physiologic, and derived data can be strengthened so that queries on any data field (e.g. male rats with > 260 g body weight) will bring up all the related studies with complete image and complementary data.
Appendix

Logarithmic Subtraction Signal and Noise Characteristics

The logarithmic subtraction signal difference and noise characteristics of this study can be written as

\[ S_{d\,(\log)} = \log(aE_o + b) - \log(aE_i + b), \text{ assuming no offset or } b = 0 \]
\[ = \log E_o - \log E_i \]

where \( E_i \) and \( E_o \) are exposures with and without contrast agent, respectively. The form \( aE + b \) uses a linear relationship of exposure to the gain (\( a \)) and offset (\( b \)) of the detector pixel value.

\[ \sigma_{S_{d\,(\log)}}^2 = \left( \frac{\sigma_{E_o}}{E_o} \right)^2 + \left( \frac{\sigma_{E_i}}{E_i} \right)^2, \text{ assuming } \sigma_{E_o} = \sigma_{E_i} \]
\[ = \sigma_{E_o}^2 \left( \frac{1}{E_o^2} + \frac{1}{E_i^2} \right) \]
\[ = \sigma_{E_o}^2 \left( \frac{1}{E_o^2 (1 - C)^2} + 1 \right), \text{ where } C = \frac{E_o - E_i}{E_o} \]

Thus, the noise contribution from logarithmic subtraction is related to the non-contrast exposure signal and the radiographic contrast, \( C \). The \( Sd\text{NR}^2 \) of logarithmic subtraction is expressed as:

\[ Sd\text{NR}^2_{(\log)} = \frac{\left( \log \frac{E_o}{E_i} \right)^2}{\sigma_{E_o}^2 \left( \frac{1}{E_o^2} + \frac{1}{E_i^2} \right)} = \frac{2 \log \frac{E_o}{E_i}}{\sigma_{E_o}^2 \left( \frac{1}{E_o^2 (1 - C)^2} + 1 \right)}, \text{ where } C = \frac{E_o - E_i}{E_o} \]
LabVIEW Code

Monitor

Purpose
The monitoring system can acquire up to 16 signals in real-time for analog to digital conversion, calibration, derived calculations, display, and storage.

Significance
A critical feature in this system is the output of signals to the sequencer that allow for physiologic driven imaging acquisition. This allows for cardio-ventilatory gated imaging and the minimization of motion related image artifacts.

Methods
Front Panel
The user panel shows three main plots: real-time, interval, and long term data. In the real-time plot, signals (commonly the ECG and its corresponding QRS TTL) are displayed in real-time. This provides immediate feedback of responses to signals. For example, the ECG electrodes often times need their placement to be adjusted. Any adjustments will be seen in real-time and thus offer immediate feedback for faster and more reliable signal manipulation. The interval data display shows all of the signals (16 in total) over a user specified time interval (usually 5 seconds). This offers the ability to view and measure signal dependencies and time course relations. For example, to ensure proper placement of arterial catheters, the ECG is plotted alongside the blood pressure (BP) curve read at the catheter. The arterial line is properly placed when the BP curve shows the correct shape and follows the time course as the ECG. There should be a rise in BP immediately after the QRS of the ECG because the heart enters systole. During diastole,
the BP curve returns to baseline values. The long term data display shows signal changes over minutes to hours. The purpose here is to plot long term trends. For example, the heart rate could be slowly climbing (10–20 beats per minute) every 10 minutes. This could indicate that the rodent is becoming light on anesthesia and thus a higher concentration of anesthetic should be given to maintain proper levels of anesthesia.

The monitoring software also calculates derived signals such as the heart rate, inspiration flow and pressure, mean arterial pressure, and body temperature. This software can also record the data in the long term display (Long Term Data Save), and the interval display along with derived signals with up to 500 samples per second (Short Term Data Save). The outputs are tab delimited files with numerical increments (i.e. short_term-001.txt, short_term-002.txt, etc.). The saved data can be used for post-processing and data mining in combining physiologic measurements with imaging data. This feature was extensively used to record and match the blood pressure changes with the x-ray images for the drug study.
Operational Flow Chart

1. Initialization
2. Signal Input
3. Calibration
   - Short-term Display
   - Interval Display
   - Long-term Display
4. Data Save
Ventilator

Purpose
The ventilator system provides the signals to the ventilator hardware for inspiration, expiration, and breath holds.

Significance
The rodents in these studies are anesthetized due to the surgical nature of catheter placement and to reduce motion. Because of the level of anesthesia, the rodents need to be mechanically ventilated for proper physiologic function. In addition to the basic functions of inspiration and expiration, the ventilator can suspend breathing at full inspiration or at end expiration. This suspension in breathing allows for mitigation in ventilatory related motion artifacts. In addition, there is a TTL output signaling the beginning of each breath that can be used to trigger the imaging acquisition. This allows for repeatable imaging at various ventilatory cycles.

Methods
Front Panel
The ventilator software accepts input values for the number of breaths per minute and the timing control (duration of inspiration/expiration and the amount of time separating each). In addition, there are inputs for the breath hold duration. The program first operates under the timing control values. When a new timing sequence, breath rate, or breath hold is initiated, the program implements the new values at the next breath. After the breath hold has expired, the ventilator returns to its original, pre-breath hold, operating timing control values. There are three types of breath holds: 1) full inspiration (end inspiration hold button), 2) full expiration-open atmosphere (long exhale button),
3) full expiration–closed atmosphere (end expiration hold button). For the full inspiration breath hold, both the inhale and exhale solenoids are closed after a full inspiration. This will keep the lungs fully inflated. For the full expiration–open atmosphere, the inhale solenoid will be closed and the exhale solenoid will be open to the atmosphere. This will allow equalization of the respiratory exchange ratio during a breath hold. After a full exhale, both the inhale and exhale solenoids are closed for the full–expiration–closed atmosphere setting. This ensures a complete seal between the lungs and the environment.
### Timing Control

<table>
<thead>
<tr>
<th>Percent</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhale Delay %</td>
<td>0.1</td>
</tr>
<tr>
<td>Exhalation Delay %</td>
<td>26</td>
</tr>
<tr>
<td>Trigger Delay %</td>
<td>0.1</td>
</tr>
<tr>
<td>Inhale Duration %</td>
<td>22</td>
</tr>
<tr>
<td>Exhalation Duration %</td>
<td>73.9</td>
</tr>
<tr>
<td>Trigger Duration %</td>
<td>5</td>
</tr>
</tbody>
</table>

### Breath Hold

- **End**
  - Inspiration
  - Hold
  - Breath Hold Duration (s): 8
  - Breath Hold Delay (s): 0.26
  - New Hold Duration (s): 8.26
- **Breaths Held**: 8
- **Rounded Breaths Held**: 8

### Long Exhalation

- **Long Exhalation Time (s)**: 8

### Additional Settings

- **Reset Rate**
- **Breaths/min**: 60
- **Time/breath (s)**: 1
Operational Flow Chart

1. Initialization

2. Timing Input

3. Execute Inspiration and Expiration Sequences

4. New Timing Input?
   - Yes (i.e. Breath Held)
   - No
Sequencer

Purpose
The sequencer uses the outputs from the monitoring program (TTLs after processing physiologic signals) to drive the image acquisition and x-ray contrast agent injection. Essentially, the injection, exposure, and camera read are synchronized to the breathing and heart rate.

Significance
The sequencer uses the outputs of the monitoring system to drive the image acquisition and contrast agent injection. This physiologic driven image acquisition allows for very repeatable imaging and injection at certain time points in the cardio-ventilatory cycles. These sequences are what are called biological pulse sequences. They are analogous to the encoding sequences used in magnetic resonance imaging, but here a time sequence of 2D images are captured from which vascular anatomical information and blood flow information can be measured.

Methods
Front Panel
The sequencer is made up of a collection of TTL pulses that operate at different times depending on timing schemes and triggering inputs. Below is a description for a typical biologic pulse sequence for measuring cardio-pulmonary blood flow. First, the x-ray tube is prepped (counters 2 & 6). Then, the ECG signal from the monitoring program goes into counter 4 and the output (TTL) comes out of counter 3 at the QRS. If fewer than 10 pre-contrast injection images (these make the mask from which the post-contrast agent injection images are subtracted from) were acquired (counter 0),
additional cardiac-gated x-ray images are gathered. If there are more than 10 pre-contrast images, one x-ray contrast injection (counter 5) is made and then cardiac gated x-ray exposures and image acquisitions (counter 1) are repeated for each heartbeat thereafter.
Operational Flow Chart

X-ray tube prep (counters 2 & 6)

ECG input (counter 4) & amplification (counter 3)

Is this the QRS?

Yes

Has 10 pre-contrast x-ray images been acquired? (counter 0)

Yes

Inject x-ray contrast agent (counter 5)

No

X-ray exposure & camera read (counter 1)

No

Has 1 injection of x-ray contrast agent been made?

Yes

X-ray exposure & camera read (counter 1)
**MATLAB Code**

**Digital Subtraction Graphical User Interface & Archiver**

**Purpose**
The DSA GUI converts the x-ray image files from a proprietary format to raw data, then logarithmically subtracts the images, and finally prepares the result for data archiving. The Archiver directs copying the subtracted images and physiologic information to the laboratory database for storage and online distribution.

**Significance**
The DSA GUI provides a way to visually inspect the images that are used for creating the mask and thus makes the resulting subtraction free of artifacts due to spontaneous motion or dropped frames. The Archiver directs copying the DSA images and physiologic information to the laboratory database for storage and online distribution. This allows for data storage security, and sharing the image and physiologic data with collaborators.

**Methods**

**DSA GUI Operation Instructions:**
The manual selection (the first 10 images per run) of what makes up the mask image allows for the construction of a mask free of obvious errors. For example, images that have clear motion artifacts, and images that were dropped by the camera are eliminated in the mask creation. The selected images are averaged to create a single mask image. Because the noise in x-ray imaging is uncorrelated to each other between images (Poisson statistics), averaging the images reduces the noise. Of great importance here is that the images to be averaged show the rodent in the same anatomical position. This is
the main reason for the physiologic driven image acquisition and is what makes this system unique. Once the mask is created, the GUI automatically performs the subtraction, creates the appropriate file directory structure, and header files that organize the data for the Archiver. Directions for operating the GUI is below:

Place *.seq files from X-ray detector computer in G:\DSA\yearDSA\OriginalRaw

Open MATLAB

Set path to G:\

In command window, type “microCTgui.m”, and then press the “Enter” key

The microCTgui should open. The highlighted fields denote information for X-ray DSA application. The other fields are for CT data processing.

Fill in all highlighted fields.

Using mouse, select “Separate”

After this process is finished, select “Mask”

Choose the images that will be averaged for the DSA mask.

Subtracted data will be placed in G:\bb_archive\subproject_code\run_number.

Output file type is Big Endian.

The subscripts after the run_number folder refer to the type of subtraction.

No subscript → un subtracted data 16-bit signed

a → logarithmic subtraction 32-bit real

b → direct subtraction 16-bit signed

c → logarithmic paradox subtraction 32-bit real

d → direct paradox subtraction 16-bit signed

The directly subtracted images (DSA = A – B) are for viewing only. Each folder contains a header text file that gives a description of the experiment.
Outputted filename directory structure example

(pre-subtracted images)

X12345

  X12345.headfile
  X12345.0001.raw
  X12345.0002.raw

  :

(log subtracted images)

X12345a

  X12345a.headfile
  X12345a.0001.fp32
  X12345a.0002.fp32

  :

(direct subtracted images)

X12345b

  X12345b.headfile
  X12345b.0001.raw
  X12345b.0002.raw

  :

(log paradox subtraction)

X12345c

  X12345c.headfile
  X12345c.0001.fp32
  X12345c.0002.fp32

  :
(direct paradox subtraction)

X12345d

X12345d.headfile

X12345d.0001.raw

X12345d.0002.raw

:

**Header file descriptor contents example**

This file is created within each image directory. It describes the x-ray technique, animal specifics, contrast injection, study number, heart rate, and other fields of interest that describe the study. The field codes were created to be as self descriptive as possible. Dimensions are in millimeters, milliseconds, and microliters for the appropriate fields.

**Field Codes:**

<table>
<thead>
<tr>
<th>Field Code:</th>
<th>Comments:</th>
</tr>
</thead>
<tbody>
<tr>
<td>U_civmid=mdl</td>
<td>internal laboratory imaging ID</td>
</tr>
<tr>
<td>U_date=050819</td>
<td>experiment date YYMMDD</td>
</tr>
<tr>
<td>U_code=03.mdl.02</td>
<td>internal laboratory imaging ID</td>
</tr>
<tr>
<td>U_specid=050819-1:0</td>
<td>internal laboratory specimen ID</td>
</tr>
<tr>
<td>U_runno=X00660a</td>
<td>imaging run number</td>
</tr>
<tr>
<td>U_parent_runno=X00660</td>
<td>imaging base run number</td>
</tr>
<tr>
<td>U_img_format=fp32</td>
<td>file type</td>
</tr>
<tr>
<td>U_headfile_db_insert=xraysub</td>
<td>internal laboratory imaging ID</td>
</tr>
<tr>
<td>U_species=Rat</td>
<td>species</td>
</tr>
<tr>
<td>U_modality=MicroX-Ray</td>
<td>modality</td>
</tr>
<tr>
<td>U_focus=chest</td>
<td>imaging FOV</td>
</tr>
<tr>
<td>U_orient=supine</td>
<td>rodent orientation</td>
</tr>
<tr>
<td>U_anatplane=cor</td>
<td>imaging plane</td>
</tr>
</tbody>
</table>
xray_height=1072  
image pixel height
xray_width=350  
image pixel width
xray_no_images=27  
number of images after subtraction
xray_tube_type=W tube Philips SRO-0950  
x-ray tube type
xray_energy=70  
x-ray kVp
xray_current=400  
x-ray mA
xray_time=10  
x-ray ms
xray_SOD=695  
x-ray source to object distance
xray_SDD=720  
x-ray source to detector distance
xray_filter=None  
x-ray filter type
xray_binning=2  
detector binning (1x1, 2x2, 4x4, etc.)
xray_camera=ImageStar  
detector type
xray_camera_pitch=0.05  
detector pixel pitch
xray_FOV_height=107.2  
image height (mm)
xray_FOV_width=35  
image width (mm)
xray_catheter_location=jugular vein  
contrast injection site
xray_contrast_agent=Isovue370  
contrast agent type
xray_contrast_inj_time=50  
contrast injection duration (ms)
xray_contrast_inj_volume_ul=50  
contrast injection volume (µL)
xray_contrast_inj_sequence=One Injection  
number of injections
xray_ECG_RtoR=156  
ECG R-R interval (ms)
xray_image_acq_delay_from_QRS=0  
imaging delay (ms) from ECG QRS
xray_contrast_inj_delay_from_QRS=0  
injection delay (ms) from ECG QRS
xray_vent_gating=Suspended End Expiration  
type of ventilatory breath hold
xray_subtraction=Log Standard  
type of digital subtraction
Archiver Operation Instructions:

Once the above DSA GUI has finished, the Archiver will direct copying the DSA images and physiologic data onto the laboratory storage and distribution center. The Archiver is a simple program that creates a TXT file describing the number and location of the images. A recurring script operating from the laboratory storage center searches for these TXT files and once found, uses the information within the TXT file to locate, copy, and distribute the images.

Open MATLAB

Set path to G:\

Archive(runno1:runno2)

This will create a TXT file describing the location and number of images for all the DSA datasets with run numbers between runno1 and runno2.
SVD Based Flow Metric Calculations

Purpose
The SVD based flow metric calculation computes the relative blood volume (PBV), calibrated pulmonary blood flow (PBF), and mean transit time (MTT) from the x-ray images at specific regions of interest.

Significance
The SVD based flow metric calculations are a fundamental piece of this work in measuring cardio-pulmonary blood volume, flow, and mean transit time. The SVD based method in terms of theory and corrections (bolus delay and vessel thickness) are described in the chapters above.

Methods
1) DSA images are median filtered to reduce the salt and pepper x-ray image noise.
2) ROIs are selected that correspond to the input function and distal regions of interest where the flow metrics are to be calculated
3) SVD performed, elements in the W matrix from SVD is thresholded and the max residue function is taken resulting in the relative flow rate (rPBF).
4) The relative blood flow information is corrected for bolus delay errors and vessel thickness modulation. Finally, the corrected relative flow rate is calibrated to absolute units (mL/min) by scaling it to the thermodilution or Fick scaling constants.
5) PBV and MTT are then calculated.
References


23. S. Ono, V. Bhargava, L. Mao, G. Hagan, H. A. Rockman and J. Ross, Jr., "In vivo assessment of left ventricular remodeling after myocardial infarction by digital


Biography

EDUCATION AND AWARDS

Duke University, Durham, NC, September 2008
Ph.D. in Biomedical Engineering (Imaging/Electrical Concentration)

- Duke University Engineering Fellowship (covers full tuition & stipend for entire education)
- Duke University Preparing Future Faculty Fellow (One of 28 graduate students selected at Duke to observe and experience faculty responsibilities at a variety of academic institutions with varying missions, diverse student bodies, and different expectations for faculty.)

Rensselaer Polytechnic Institute, Troy, NY, December 2001
B.S. in Biomedical Engineering (Electrical Concentration) & Minor in Electrical Engineering

- Graduated 2nd in major
- Cum Laude
- Dean’s List
- Founder of Alpha Eta Mu Beta Biomedical Engineering Honor Society at RPI
- President of RPI Biomedical Engineering Society Chapter
- Rensselaer Alumni Scholarship
- Warren Point Square Club Merit Award
- Tau Beta Pi Engineering Honor Society
- Valley Hospital Auxiliary Scholarship
Peer-Reviewed Publications


Full Length Abstracts, Conference Proceedings, and Presentations


