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TECHNICAL REPORT

A new design for high stability pressure-controlled ventilation for small animal lung imaging

M.J. Kitchen,[a,1] **A. Habib,**[a,2] **A. Fouras,**[b] **S. Dubsky,**[b] **R.A. Lewis,**[a,c] **M.J. Wallace**[d] **and S.B. Hooper**[d]

[a] *School of Physics, Monash University, Clayton, Victoria, 3800, Australia*
[b] *Division of Biological Engineering, Monash University and Fluids Laboratory for Aeronautical and Industrial Research, Monash University, Clayton, Victoria, 3800, Australia*
[c] *Monash Centre for Synchrotron Science, Monash University, Clayton, Victoria 3800, Australia*
[d] *Department of Physiology, Monash University, Clayton, Victoria 3800, Australia*

E-mail: Marcus.Kitchen@sci.monash.edu.au

ABSTRACT: We have developed a custom-designed ventilator to deliver a stable pressure to the lungs of small animals for use in imaging experiments. Our ventilator was designed with independent pressure vessels to separately control the Peak Inspiratory Pressure (PIP) and Positive End Expiratory Pressure (PEEP) to minimise pressure fluctuations during the ventilation process. The ventilator was computer controlled through a LabVIEW interface, enabling experimental manipulations to be performed remotely whilst simultaneously imaging the lungs *in situ*. Mechanical ventilation was successfully performed on newborn rabbit pups to assess the most effective ventilation strategies for aerating the lungs at birth. Highly stable pressures enabled reliable respiratory gated acquisition of projection radiographs and a stable prolonged (15 minute) breath-hold for high-resolution computed tomography of deceased rabbit pups at different lung volumes.

KEYWORDS: Gas systems and purification; Multi-modality systems; X-ray radiography and digital radiography (DR)

[1] Corresponding author.
[2] Current address: Solid State Physics, University of Siegen, Walter-Flex Strasse 3, D-57068, Siegen, Germany.

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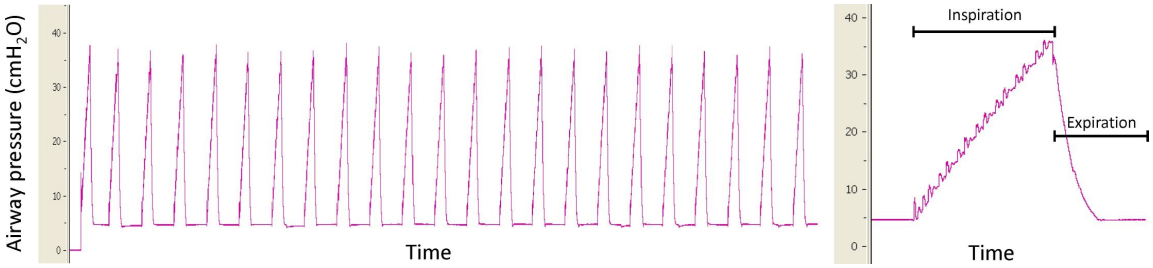
1 Introduction

The most appropriate methods for resuscitating and ventilating newborn infants at birth are largely unknown, particularly for infants born prematurely [1, 2]. This is primarily due to a poor understanding of how the lungs aerate at birth and the factors that regulate this process [3]. Before birth, the fetal lungs are liquid-filled and at birth the airways must be cleared of liquid to allow the entry of air before pulmonary gas exchange can begin [3, 4]. However, infants born very preterm usually fail to establish effective gas exchange and require respiratory support. As trans-pulmonary pressures generated during inspiration are important for airway liquid clearance at birth [5, 6], respiratory support that maintains a pressure gradient across the airway wall is essential. However, as the transition from liquid to air-filled airways greatly alters the resistance, time constant and compliance of the lung [7], the pressures, airflows and inspiratory times required to ventilate the lung are very dynamic, requiring accurate and variable control. Indeed, before the lung is fully aerated, long inspiratory times which overcome the long time constant of the water-filled airways is considered to be important, as is the application of an end-expiratory pressure [6]–[8].

Our goal is to identify the specific components of mechanical ventilation that facilitate lung aeration and are protective of the immature lung immediately after birth. Previously, we have used synchrotron-based phase contrast X-ray imaging (PCXI) to explore the factors regulating lung aeration at birth. In particular, the ability of different ventilation strategies to effectively and uniformly ventilate the neonatal lung have been examined [5]–[10]. However, to assess these strategies, a ventilator that can vary all of the different aspects of inspiration and expiration in a consistent and stable manner is required. In addition, the ventilator must be able to be controlled remotely as the imaging is conducted in a lead-lined enclosure that must be vacated whilst the X-ray beam is operational [11]; therefore, direct access to the ventilator is not possible.

We have constructed and tested a small animal ventilator that enables very stable, pressure controlled ventilation and provides greater flexibility over all ventilation parameters (e.g. inspiration/expiration flow rates, inspiratory and expiratory pressure wave forms and plateaus) than is



(a) (b)

Figure 1. (a) Airway pressure trace produced from a commercial (air pump controlled) small animal ventilator. (b) A magnified segment of a single ventilation cycle, revealing an almost linear increase in pressure contaminated by oscillations in the pressure waveform.

possible with most commercially available ventilators. Our system also provides synchronisation between the ventilator and image acquisition, so that different methods of ventilation may be compared in order to provide a greater understanding of the neonatal ventilation process.

1.1 Ventilation in small animals

Most commercially available ventilators for small animals either utilise a piston to deliver airflow or an air pump that delivers air at a predetermined flow rate until a set positive inspiratory pressure (PIP) is reached (figure 1). Piston driven ventilators regulate inspiratory gas flow rates by regulating the speed of the piston and limit the PIP using either a pressure release valve or feedback control of the piston speed. However, it is difficult to regulate the inspiratory pressure waveform (figure 1(b)) and lengthy end-inspiratory pauses at a constant pressure are problematic. Pauses during the respiratory cycle can be important for high resolution imaging, particularly at end-inspiration, to avoid motion blur. Dynamic changes in lung compliance alter the pressure/volume relationship and the need to move the piston to generate flow makes it difficult to maintain a stable airway pressure, particularly in a partially aerated lung in which the compliance is rapidly changing.

Ventilators that use an air pump to generate airflow usually limit the PIP by opening the expiratory valve once the PIP is reached or have a pressure-release valve. Thus, the inspiratory time can be truncated if the flow is high and the PIP is reached before the preset inspiratory time. If the flow is lowered to maintain a constant inspiratory time, the PIP achieved is not constant and rarely reaches the set PIP. Furthermore, a post-inspiratory pause at constant pressure is not possible. On the other hand, pressure release valves, particularly if they are spring activated, can lead to oscillating airway pressures and pressure spikes that are potentially injurious to the lung. Furthermore, most ventilators generate a positive end-expiratory pressure (PEEP) by placing the end of the expiratory line at a set depth below the surface of a water-filled container. The resulting bubble formation can initiate pressure waves that are transmitted to the lung, which can add to motion blurring during high resolution image acquisition.

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2 Instrumentation and methods

2.1 New ventilator design

We have developed a time-cycled pressure-limited ventilator that is controlled through a personal computer running a custom designed LabVIEW (National Instruments, Austin, Texas) Virtual Interface (VI). The system enables the synchronization of image acquisition with mechanical ventilation of small animals. A schematic diagram demonstrating how air is cycled around the ventilator and delivered to the lung is displayed in figure 2. Two pressure vessels are used to control the PIP and PEEP. Although fed by a single air pump the vessels stored air at two independent pressures. At the start of inspiration, the inspiratory solenoid valve (Cole-Parmer, EW-01367-50) opens whilst the expiratory solenoid remains closed for the set inspiratory time. Air from the PIP vessel flows to the lung through the inspiratory solenoid via a variable restrictor valve (Aalborg, SMV40-S); this allows air to flow into the lungs until the airway pressure reaches the pressure of the PIP vessel. The PIP and PEEP vessels are relatively large in volume ($\sim$1 L/box) and the gas flows into and out of them are much greater than the inspiratory and expiratory flows to and from the animal. This minimizes the volume and pressure changes associated with opening and closing of the respiratory solenoids and enhances the stability of the applied PIP and PEEP pressures. An electronically controlled variable restrictor valve (Aalborg, SMV40-S) allows analog variability of the rate of gas flow into the lung from the pressure vessel. As a result, the inspiratory pressure wave can be varied and the length of an inspiratory pressure plateau (as a proportion of inspiration time) can be set by regulating the inspiratory airflow independently of the PIP. Upon expiration, the states of the respiratory solenoids are simultaneously inverted, allowing the lungs to deflate to the lower PEEP level for a preset period. During expiration, airflow from the lung into the PEEP box can also be regulated via a restrictor valve, as shown in figure 2. Both the inspiratory and expiratory times can be adjusted while the ventilator is operating. As a safety precaution, both solenoids simultaneously close when the ventilation sequence is terminated to prevent over-distension of the airways.

The ventilator is controlled using a National Instruments data acquisition module (NI USB-6259) via the LabVIEW interface, as illustrated in figure 3. The data acquisition system is connected to the PC via a Universal Serial Bus (USB) port and can control up to 4 analog outputs, 80 analog inputs and 48 digital input/output channels at 1.25 MS/s. LabVIEW can synchronously control multiple devices and readily interface with external hardware. The main advantage of the virtual interface is that all parameters (e.g. air pressure and flow rate) can be controlled remotely with a real-time display.

In studies focused on imaging and ventilation, it is important that the lung inflates uniformly to a constant pressure to provide consistency in lung inflation from breath to breath. Since the inflation pressure is provided by simply opening the inspiratory solenoid and exposing the lung to the pressure within the PIP vessel, the inspiratory pressure cannot exceed the pressure in the PIP vessel, thereby eliminating the danger of exposing the lung to large pressure spikes. Furthermore, as the PIP and PEEP vessels have a capacity of $\sim$100 times the inspired volume, pressures in the PIP and PEEP vessels remained stable throughout ventilation, allowing airway pressures to remain fixed indefinitely; this feature was utilized in computed tomography (CT) scans of deceased rabbit pups at different airway pressures. Although these pressure vessels increase the overall size of the system, the stability in airway pressure they provide far exceeds any concerns relating to ventilator size.

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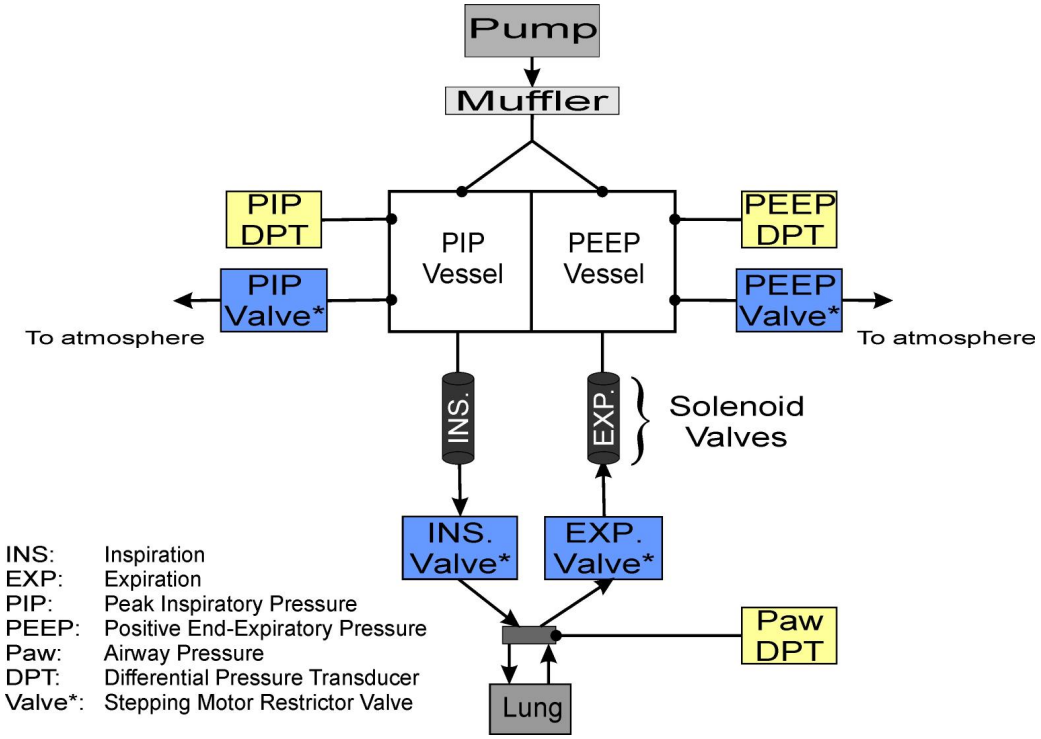

Figure 2. Schematic representation of air flow through the ventilator. Arrows indicate the direction of the flow, which is generated by a gas pump.

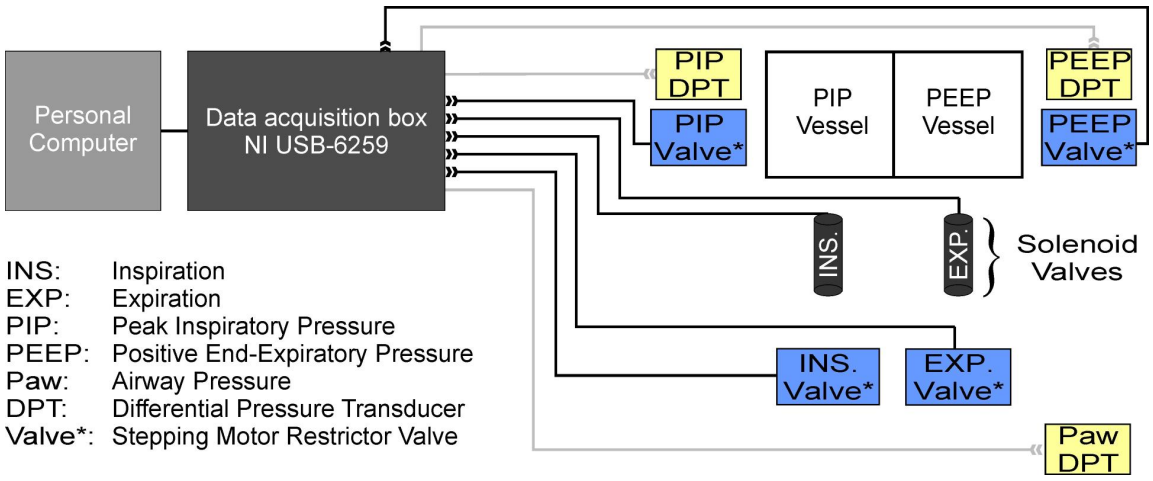

Figure 3. Schematic diagram of the electrical wiring between components of the ventilator and the data acquisition system. Black lines represent outputs and grey lines represent inputs from the data acquisition system.

The pump supplying air into the PIP and PEEP pressure vessels is connected in line with a muffler to dampen the pump-generated pressure waves. As mentioned, air pressure and flow to the pressure vessels and to the lung are controlled by variable (stepping motor) restrictor valves (Aalborg, SMV40-S). The speed of the stepper motors is controlled using LabVIEW via a logarithmic

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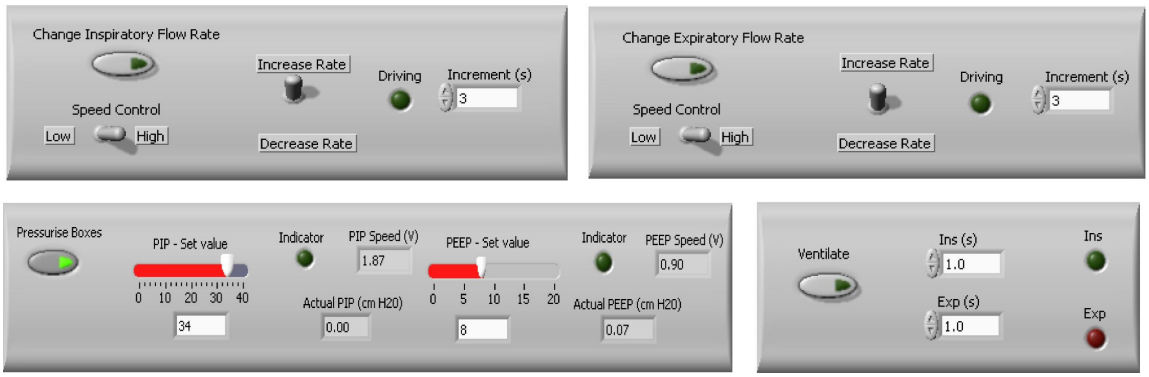

Figure 4. Virtual interface of the ventilator. (Table 1 describes the features of the interface).

function of motor speed against the difference between the set and measured pressures. Thus, the bigger the pressure difference between set and measured pressures, the faster the stepper motor moves and vice versa. The asymptotic logarithmic function ensures the motor speed is limited to prevent over-shoot of the required pressure to protect the lungs against sudden pressure rises. Motor speeds are updated every 100 ms after sampling the pressure in the PIP and PEEP vessels. Sampling faster than this occasionally led to communication errors between the LabVIEW and the data acquisition system.

Pressure in the PIP and PEEP vessels was measured via differential pressure transducers (Ashcroft DXLdp) connected to the data acquisition system (figure 3). The pressure transducers were chosen so that the pressures measured fell within the linear region of their response curves. Before each experiment the pressure transducers were calibrated using a digital manometer and a two-point calibration; these transducers showed negligible signal drift at a constant pressure for the duration of the experiments and their calibration typically varied by less than 1% between experiments. The virtual interface (figure 4) displays the calibrated pressure readings and enables regulation of the pressures within the vessels by activating the relevant stepper motor control flow valves that vented the vessels to atmosphere. If the pressure is too high then the relevant valve widens to allow more air to flow to atmosphere; conversely if the pressure is too low the valve restricts flow to atmosphere. The VI enables the desired PIP and PEEP settings to be adjusted while the ventilator is operational.

Tubing dimensions throughout the ventilator have been optimised to stabilize the air flow and pressure to each component. For example, large diameter tubing (exhibiting low resistance to flow) is connected to the input of the higher pressure (PIP) vessel with higher resistance tubing connected to the lower pressure (PEEP) vessel. The airway pressure at the mouth opening is recorded using another pressure transducer.

2.2 Data acquisition

Figure 5 shows the geometry of the PCXI experiments. Changes in lung air volume were measured directly using a water-filled plethysmograph (figure 5). Plethysmography follows the Archimedean principle of volume measurement via fluid displacement; in this instance, the volume of fluid displaced by the increase in lung gas volume during inspiration. This volume was equal to the amount

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Table 1. Control panel description.

Software Feature	Task
Open Valves	Open PIP and PEEP valves to vent PIP and PEEP vessels to atmosphere
PIP and PEEP Set Value	Set the desired pressure values
Actual PIP and PEEP	Measured pressure value in cmH_2O
Pressurise Boxes	Regulate pressure in PIP and PEEP vessels
PIP and PEEP speed	Indicates the speed of stepper motor (0-5 V DC) connected to the PIP and PEEP vessels
Inspiration and Expiration	Timing (seconds) for inspiration and expiration
Increase/decrease rate	Change air flow rates to and from the lung
Speed control	Speed for valve opening
Increment	Duration (seconds) to move stepper motor to obtain desired flow rate
Ventilate	Start ventilation cycle

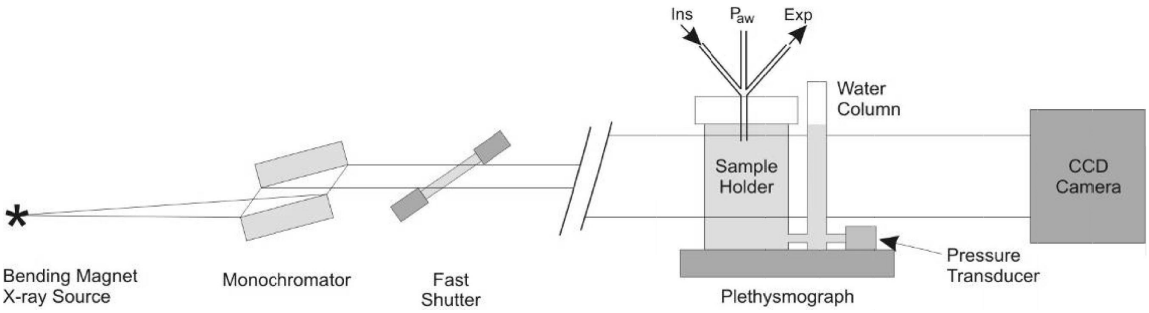

Figure 5. Schematic diagram of the geometry for propagation based phase contrast X-ray imaging, which shows the water plethysmograph used to measure lung air volume.

of water that flowed from the main chamber into a water column during each breath. A PowerLab data acquisition unit was used in conjunction with LabChart software (PowerLab/LabChart, AD Instruments, Sydney, Australia) to display, record and analyse the airway pressure, lung air volume and inspiratory/expiratory waveforms. The airway volume was calibrated using a pressure transducer by measuring the pressure increase for a known volume (1 mL) of water.

2.3 Imaging methods

Rabbit pups were mechanically ventilated and simultaneously imaged *in vivo* on beamline 20B2 in the Biomedical Imaging Centre at the SPring-8 synchrotron radiation research facility, Japan. All procedures involving the use of animals were approved by the SPring-8 Animal Care and Use Committee as well as the Monash University Animal Ethics Committee.

For live imaging experiments, pups were delivered by caesarean section, intubated via tracheotomy, and connected to the ventilator; for further details see reference [10]. The pups were

then immediately placed in a pre-warmed water-filled Perspex imaging chamber (head out) inside the experimental hutch.

A digital output from the ventilator (inspiration onset) was used to gate triggers sent to the fast X-ray shutter and CCD (Charge Coupled Device) camera during the ventilation cycle (figure 5). The X-ray shutter was employed to avoid delivering unnecessary radiation dose to the pups between exposures. A custom-designed pulse generator provided a train of 7 triggers to the fast shutter during each ventilation cycle. A Uniblitz VMM-T1 delay generator (Vincent Associates, NY, USA) sent the same pulse train, delayed by 50 ms, to the CCD camera to ensure the X-ray shutter was fully open during the exposure. The opening period of the shutter and camera activation trigger was also recorded using Powerlab to enable synchronisation of imaging data with the chart recordings.

High-resolution Computed Tomography (CT) scans were also performed for quantitative *in situ* measurements of the lung's alveolar dimensions as a function of airway pressure. For CT sequences, the pups had been humanely killed shortly after completing the live imaging experiments. Minimising motion blur was critical for a quantitative reconstruction of the minor airways. Initial experiments employed respiratory gating, whereby each rotated projection image was recorded at end expiration. However, reconstructed images revealed an unacceptable amount of motion throughout the scan due to ventilation-induced movement of the pup. Subsequent experiments were performed using a constant pressure to keep the lungs at an approximately constant volume. Due to the dynamic compliance of the lung it was essential to reduce the acquisition time to minimise detectable changes in airway morphology. A scan time of just 15 minutes was enabled by continuously rotating the pups throughout the scan. (Note that this scan time is comparatively fast for high resolution CT on this beamline, but is very slow compared with clinical CT scanners that require only a few seconds or less.) The ventilator was thus required to maintain a highly stable pressure for 15 minutes. Flat field images were recorded at the beginning and end of the CT for normalisation of the 1200 projections used for reconstruction. CT slices were reconstructed using a filtered-back projection algorithm using a Hanning filter.

For all imaging experiments, an X-ray energy of 24 keV was chosen to achieve good phase and absorption contrast [9]. A gadolinium oxysulfide (Gd_2O_2S) scintillator and tandem-lens coupled to a CCD camera (Hamamatsu C4742-95HR) with an effective pixel size of 22.47 μm was used to image the rabbit pups, which provided sufficient spatial resolution to observe the terminal airways ($\sim$120–150 μm) [9, 10].

3 Results and discussion

Rabbit pups were ventilated while sequences of images were acquired under different ventilation conditions to demonstrate the rate and spatial pattern of lung aeration. Some experiments employed a long (up to 20 s) inspiration time at the start of ventilation to facilitate airway liquid clearance and enhance aeration of the distal gas exchange regions of the lungs. This long inspiration time required a long (up to 20 s), highly stable end-inspiratory pressure plateau which was easily achieved using this ventilator, demonstrating both its flexibility and stability. Figure 6 shows an initial long inspiration period of 20 s, followed by a ventilation cycle with the inspiration and expiration times set at 1 s. During the long inspiration, the lung is pressurised to the same pressure in the PIP vessel. Figure 6 clearly illustrates that the ventilator is able to maintain an extremely stable pressure ($\pm$0.2

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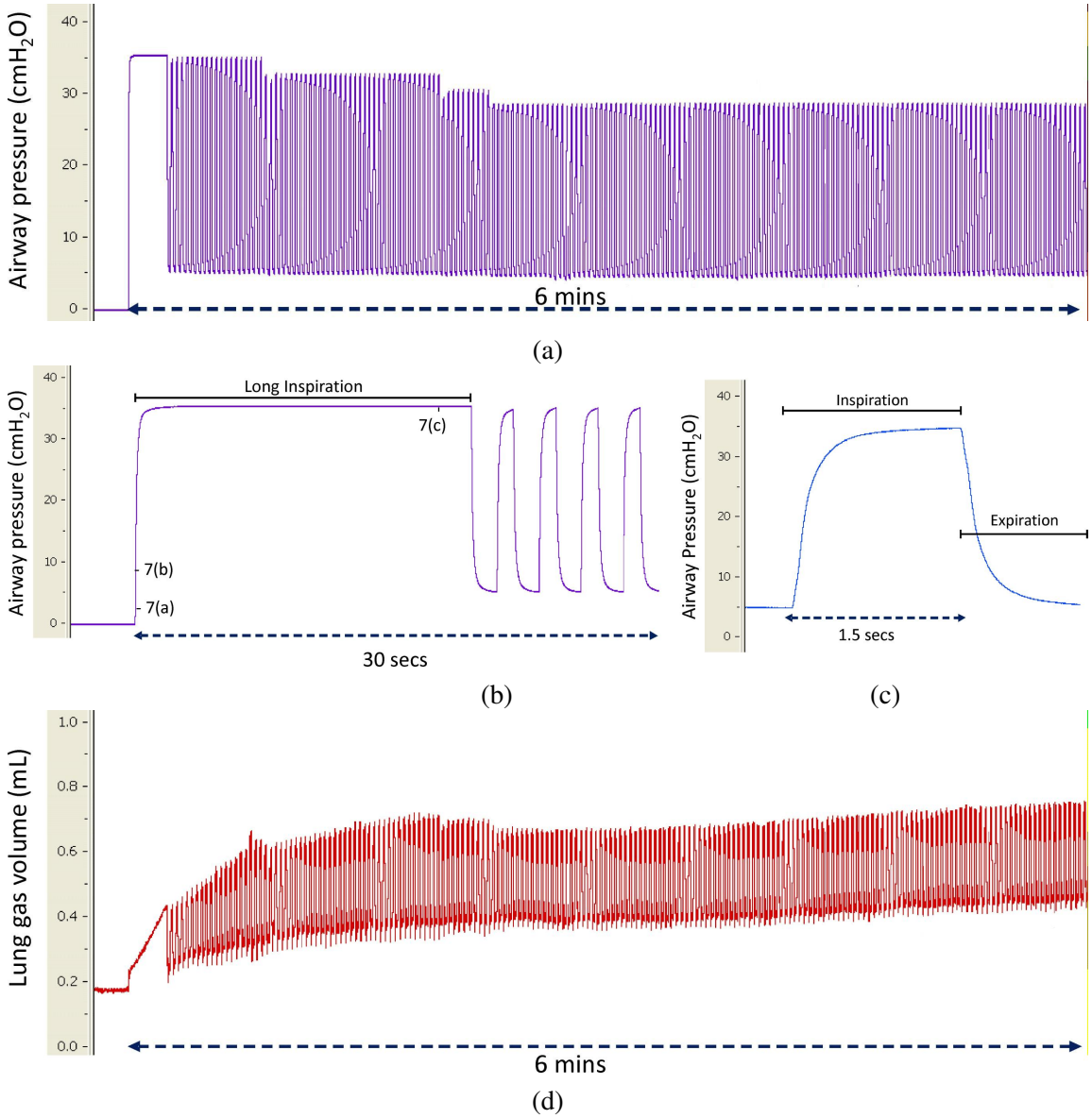

Figure 6. (a) Airway pressure recorded during the phase contrast X-ray imaging experiments. Magnified views of (a) highlight (b) the stable pressure provided during long inspiration and (c) the smooth, asymptotic pressure changes during inspiration and expiration. (d) Lung gas volume measured by the plethysmograph in response to the pressure signal shown in (a).

cmH_2O) for a prolonged period. This demonstrates the benefit of utilising vessels containing air at fixed pressures over the piston-based system whereby a piston must be constantly driven to create a pressure.

Figure 6(a) further demonstrates the stability of the ventilator before, during and after operator determined alterations in PIP. The PIP was set to 35 cmH_2O for the initial sustained inflation (20 s) and, after 19 standard respiratory cycles, the PIP was reduced to achieve an appropriate tidal volume. The PIP was then reduced twice more to a final setting of 29 cmH_2O. Upon each alteration

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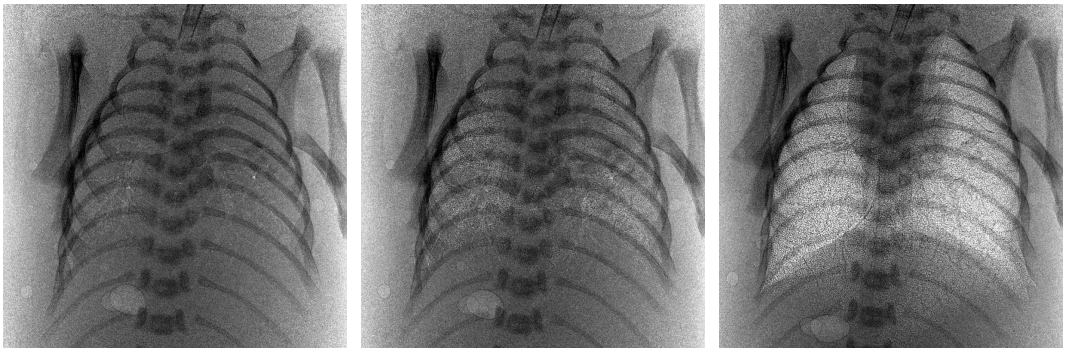

(a) (b) (c)

Figure 7. Phase contrast image of rabbit pup. (a) Fluid-filled lung; (b) reveals the major airways during the first inspiration and; (c) lungs at peak inflation. Image acquisition times are labelled in figure 6(b). Image size: 20.97×16.87 mm^2. Exposure time: 250 ms. Energy: 24 keV.

in PIP setting, the pressure trace oscillated slightly until the set pressure was reached within 3-4 breath cycles (4.5–6.0 s, depending on respiration rate).

Figure 6(d) shows the volume of air delivered to the lungs of the pup ventilated by the pressure waveform shown in figure 6(a), as measured using the plethysmograph. This figure demonstrates the ability of the ventilator to deliver a steady volume of gas to the lungs in response to the changing pressure provided by the ventilator.

Figure 7 shows images acquired during the ventilation cycle displayed in figure 6(b). At the beginning of imaging, the lungs were fluid-filled and so were not visible with phase contrast X-ray imaging (figure 7(a)). Once ventilation commenced the air moved rapidly into the smallest and most distal airways, as is evident in the appearance of the trachea, major bronchi and some of the smaller airways. In figure 7(b) the partially inflated lung and some of the airways are also visible. Figure 7(c) shows the lung at peak inflation.

Figure 8 shows an axial slice of the reconstructed CT image of a deceased rabbit pup chest, which shows the fully aerated lung. Dark grey areas represent airways, moderate grey areas represent soft tissues and the lightest grey regions represent high density bony structures. The CT reconstruction shows only minimal motion blurring despite the long acquisition time of 15 minutes. The small artifacts are likely due to slight movement of the pup as it rotates, although small changes in lung volume can occur, due to changes in lung compliance, despite the fixed pressure. A brighter X-ray source (e.g. polychromatic) and more sensitive detector would have allowed a shorter acquisition time to minimise these artifacts. The minimal motion artifacts are a testament to the remarkable stability in airway pressure maintained by the ventilator hardware.

The ventilator described in this paper is a pressure controlled device. The stability of the pressure is limited by the sampling of the pressure transducers and subsequent control of stepper motor valves from the software. Future planned improvements will include dedicated electronics for pressure control in the PIP and PEEP vessels so that the pressures can be measured at a much greater sampling rate to further improve ventilator stability. The system could also be adapted to provide volume-controlled ventilation to ventilate the lungs to a preset gas volume. This would require the development of an accurate method for measuring expired flow, which would be inte-

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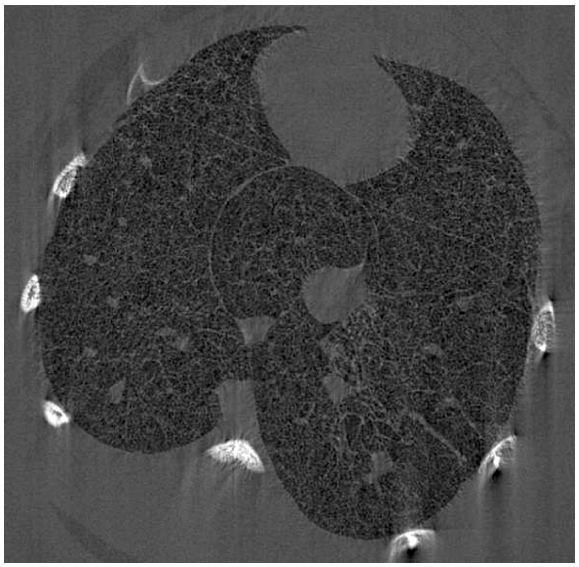

Figure 8. CT lung image of a rabbit pup. Image size: 22.51 $\times$ 21.95 mm^2. Energy: 24 keV. Exposure time per projection: 250 ms. Total scan time: 15 minutes.

grated electronically to yield volume. Controlling airway volume will eliminate any effects caused by dynamic compliance changes during prolonged inflations required for high resolution computed tomography scans.

4 Conclusions

This paper describes a new custom-designed ventilator that can ventilate small animals during PCXI experiments with a high degree of flexibility; enabling us to change the ventilator settings remotely at any stage during ventilation. Importantly, the ventilator is able to produce a highly stable pressure waveform due to the use of a fixed pressure vessel system with a volume that greatly exceeds the tidal volume. These studies will provide a greater understanding of the neonatal ventilation process, which will lead to better ventilation strategies for premature newborn infants. The stability of the ventilator was such that we could also acquire high resolution CT scans of the inflated lung for the purpose of calculating lung airway dimensions and air volumes.

Acknowledgments

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