"I do not think there is any thrill that can go through the human heart like that felt by the inventor as he sees some creation of the brain unfolding to success… Such emotions make a man forget food, sleep, friends, love, everything."
Nikola Tesla

Chapter 1. Motivation, Significance and Innovation

1.1 Introduction

Development of mouse models of human diseases has been dramatically facilitated by the recent completion of the Mouse Genome Project [1, 2] and associated mouse model research [3-7]. There are now many mouse models of human diseases available, from spontaneous disease models to genetically manipulated models allowing reproducible disease phenotypes. The increasing number of mouse models used in research has created a need for small animal imaging systems that assimilate clinical systems such as Computed Tomography, Magnetic Resonance Imaging and Positron Emission Tomography. There is now an array of small animal imaging systems including, but not limited to, micro-Computed Tomography (CT), micro-Magnetic Resonance Imaging (MRI), micro-Positron Emission Tomography (PET) and bioluminescence tomography (BLT). These systems are particularly appealing to molecular biologists as they can move from *in vitro* to *in vivo* models that better represent disease states. In addition, longitudinal imaging of biological processes is now possible, providing information regarding disease progression and their response to therapies. However, imaging small animals, such as mice, poses new problems not seen in larger animals or humans due to their small size, and, when

performed *in vivo,* their rapid cardiac and respiratory rates become a major obstacle, resulting in motion artifacts.

In this thesis, we focus on small animal imaging techniques that are relevant to pulmonary disease research. In particular, we focus on micro-CT, laser scanning confocal microscopy (LSCM) and a novel three-dimensional (3D) pathology imaging system. Micro-CT was chosen because it currently provides the highest spatial resolution three-dimensional, non-invasive imaging for small animals. In addition, it assimilates well to clinical Computed Tomography (CT), which is used for patient pulmonary disease care and hence reduces the learning curve when transitioning to small animal pulmonary imaging. The laser scanning confocal microscopy system was chosen based on its ability to be translated into an endoscopic biopsy system for human use. Also, confocal microscopy had never been used for assessment of lung structure and function in either normal or diseased lungs *in vivo*. Finally, a 3D pathology system was constructed and utilized for accurate correlation of histopathology to the *in vivo* small animal imaging systems.

Unlike their clinical counterparts, the majority of small animal imaging systems are in their infancy and far from turnkey. In practice, most applications require minor to major custom protocol and equipment development. Over time, small animal imaging systems will advance and, with upcoming second and third generation systems that are currently in development, become more like their clinical brothers. In pulmonary research, imaging the lung *in vivo* is very difficult using any imaging modality due to the organ's important micro-structural pathology and anatomical dynamics. In this work, we present several novel techniques developed and implemented to allow greater use of small animal imaging systems for pulmonary research as well as introduce new imaging techniques specifically for lung imaging.

1.2 Thesis Overview

The major body of work performed and presented in this thesis is grouped into four major chapters, each of which has been published or is in preparation for publication in major international peer-reviewed journals. This thesis begins with an

introductory background chapter, and concludes with an executive summary listing accomplishments, achievements and future direction of the presented work.

1.2.1 Chapter 2: Background

The second chapter of this thesis provides an overview of the essential concepts needed for a complete understanding of the conducted research. These background concepts include the structure and function of the lung, the development of histology and pathology as a gold standard for pulmonary disease research, the progression of X-ray imaging into micro-CT, the creation of novel microscopy imaging techniques developed from LSCM, and finally the study and understanding of human disease through mouse models. Although a thorough discussion on the background of every topic cannot be performed within this chapter, considerable detail has been given to appreciate the current state of the art and gaps that still exist.

1.2.2 Chapter 3: 3D Lung Pathology Imaging

In Chapter 3, we describe a custom microscopy system developed in house to acquire high-resolution pathology images featuring microscopic detail that can be spatially correlated to whole organ non-invasive imaging systems such as CT. A comprehensive discussion of the design and development of the Large Image Microscope Array (LIMA) system is provided, including the development of the novel large-blade vibrating microtome, integration of the 1.3 megapixel peltier cooled CCD camera, high magnification microscope and three-axis gantry. A description of the computer controlled C++ program developed to automate the entire sectioning and automated raster-scan imaging sequence is given. Tests carried out on fixed lung tissue from sheep and mice, resulting in large, high-quality image datasets with minimal distinguishable disturbance in the delicate alveolar structures, are discussed. Finally, accurate registration of pathology images acquired using the LIMA system with non-invasive micro-CT imaging is presented.

1.2.3 Chapter 4: Micro Computed Tomography Lung Imaging

Chapter four is segmented into two parts, *ex vivo* and *in vivo* imaging. In the first section, we present several techniques developed to reduce associated noise and increase the resolution and contrast of micro-CT images. These include ring artifact reduction, gantry miss-alignment and wobble correction, beam hardening correction and Hounsfield calibration. In the second section of this chapter, we present a novel breath hold technique for capturing the lung microstructure *in vivo* using the micro-CT system. We have termed this new technique Intermittent Iso-pressure Breath Hold (IIBH) gating, which essentially pauses the respiratory motion during image acquisition, thereby increasing the resolution and SNR of the reconstructed images. We compare four gating techniques, i.e. no gating, Late Expiratory (LE) gating, Late Inspiratory (LI) gating and finally Intermittent Iso-pressure Breath Hold (IIBH) gating. Quantitatively, we compare several common image analysis methods used to extract valuable physiologic and anatomic information from the pulmonary system and show that the IIBH technique produces the most representative and repeatable results.

1.2.4 Chapter 5: Laser Scanning Confocal Microscopy Lung Imaging

Chapter five describes two laser-scanning techniques developed for imaging lung tissue in a natural state. The first section describes an *ex vivo* technique developed to image intact fresh mouse lungs using a desktop LSCM. A study using this technique is presented, assessing alveolar structure over a respiratory cycle in five mice lungs. The results show, for the first time, direct evidence of alveolar recruitment / de-recruitment in the normal mouse lung. A new theory on alveolar dynamics is proposed based on the quantitative results. In the second section of this chapter, an *in vivo* imaging technique based on a fiber optic LSCM is presented. A detailed description of the imaging hardware and software used to acquire and process the catheter based confocal microscopy (CBCM) images are given. Finally, imaging of the alveoli in live, breathing mice lungs is presented with clear depiction of alveoli and supporting structures.

1.2.5 Chapter 6: Longitudinal Multi-Modal Assesment of Lung Cancer

In the sixth and final research chapter, techniques developed and described through out this thesis are utilized to undertake a longitudinal multi-modal study investigating a carcinogen induced mouse model of lung cancer.

In the first step of this study, a group of mice was treated with Urethane, a carcinogen known to induce lung tumors in mice. These mice were then scanned multiple times over a span of 6-months using the *in vivo* micro-CT technique developed in Chapter 4. This longitudinal data provided a means for accurate quantitative evaluation of tumor growth profiles over time within and across mice. As a pilot study, a subset of mice was also imaged at the 6-month point using a micro-MRI and at the 6 & 9-month points using micro-PET imaging for both additional structural and functional information. Mice were finally imaged using the *in vivo* CBCM and *ex vivo* LSCM technique outlined in Chapter 5. Here, select normal, suspicious and tumor regions were imaged at the cellular level on each mouse lung. The lungs were then fixed, externally supported using a custom foam technique and re-scanned on the micro-CT scanner. Fixed supported lungs were then imaged and sectioned on the LIMA system as described in Chapter 3. Slices from the LIMA sectioning were processed for H&E histology.

A series of computer registration techniques were then implemented in order to align the *in vivo* micro-CT datasets to their respective fixed lung *ex vivo* micro-CT, LIMA and subsequent histology datasets. This resulted in an accurately registered cascading dataset from the *in vivo* micro-CT images down to the "ground truth" histology.

Tumors were identified and tracked over time using the *in vivo* micro-CT datasets, and growth rates were traced back to their respective underlying histology acquired at each end-point. Finally, the utility of the CBCM technique for early diagnosis of lung cancer was investigated through analysis of normal, suspicious and tumor tissue acquired throughout this longitudinal mouse lung cancer study.

1.3 Conclusion

In the series of projects presented in this work, a framework for imaging mouse lung pathology with particular emphasis on lung cancer from its inception and spatially correlating the cellular makeup to non-invasive imaging techniques, has been accomplished. In order to achieve this goal, an array of novel imaging techniques has been developed and implemented for imaging mouse lungs undergoing carcinogenesis. The overall application of the thesis is the use of novel imaging techniques for understanding lung structure and function and, in particular, the detection, diagnosis and understanding of lung cancer in its early phase, a problem that has plagued lung cancer research to date. The techniques presented have been developed with future translation into the clinical setting as the goal. In particular, the fiber-optic catheter based confocal microscopy system was developed for translation into clinical bronchoscopy procedures in the form of an optical biopsy tool. As an accumulation of the techniques developed throughout this thesis, a longitudinal mouse lung cancer study has been carried out. This study incorporates the use of the developed *in vivo* micro-CT system, *in vivo* LSCM system and 3D pathology system, all of which are described within this thesis.

1.4 Statement of Original Contributions

The following bullet list is a compilation of the original contributions made by the candidate towards the work presented in this thesis.

Chapter 3: 3D Lung Pathology Imaging

- Design and construction of large-scale vibrating knife microtome
- Motorization and automation of Leica 2500M microtome
- Design and construction of 3-axis motorized gantry
- Design and construction of photo-locking mechanism
- Design and development of automation software using NI Labview

Chapter 4: Micro Computed Tomography Lung Imaging

- Design and construction of micro-CT geometry and beam hardening phantoms
- Coding and implementation of dynamic source to detector correction algorithm using Matlab and EXXIM Cobra software
- Design and implementation of dynamic center offset correction algorithm using Matlab and EXXIM Cobra software
- Coding and implementation of ring artifact correction algorithm using Matlab
- Implementation of beam hardening correction algorithm using the EXXIM Cobra software
- Analysis of geometry and beam hardening correction algorithms
- Design and development of the *in vivo* Intermittent Iso-Pressure Breath-hold technique
- Design and development of the respiratory gating software using NI Labview
- Development and execution of the animal imaging protocols and breath-hold comparison study

Chapter 5: Laser Scanning Confocal Microscopy Lung Imaging

- Design and development of the *ex vivo* mouse lung confocal imaging technique

- Design, prototyping and development of the *ex vivo* lung imaging chamber
- Design, prototyping and development of the microcomputer pressure controller
- Design and execution of the whole lung alveolar mechanic study
- Design and implementation of the automated alveolar morphometric analysis software coded in Matlab
- Conception of the pores of Kohn theory
- Design and construction of the *in vivo* catheter-based confocal microscope attachment and catheter probe
- Design and implementation of the fiber optic image processing and analysis software developed in Matlab
- Design and execution of the *in vivo* mouse lung imaging study

Chapter 6: Longitudinal Multi-Modal Assessment of Lung Cancer

- Design and development of the micro-CT heating chamber
- Design, development and construction of the flexible miniature mouse bronchoscope
- Design, development and construction of the portable breath-hold micro-controller
- Development and execution of the longitudinal animal handling, anesthesia and imaging protocols for the micro-CT and LSCM.
- Design and execution of the A/J Urethane mouse lung cancer study
- Design and development of the polyurethane whole lung foam embedding technique
- Development and implementation of the multi-modal mouse lung LIMA imaging procedure
- Development and implementation of the multi-modal mouse lung registration procedure in Image J and AMIRA software package
- Segementation and analysis of the lung tumor growth rates
- Development of the mouse lung tumor labeling system
- Development and implementation of the confocal microscopy fluorescent labeling procedures