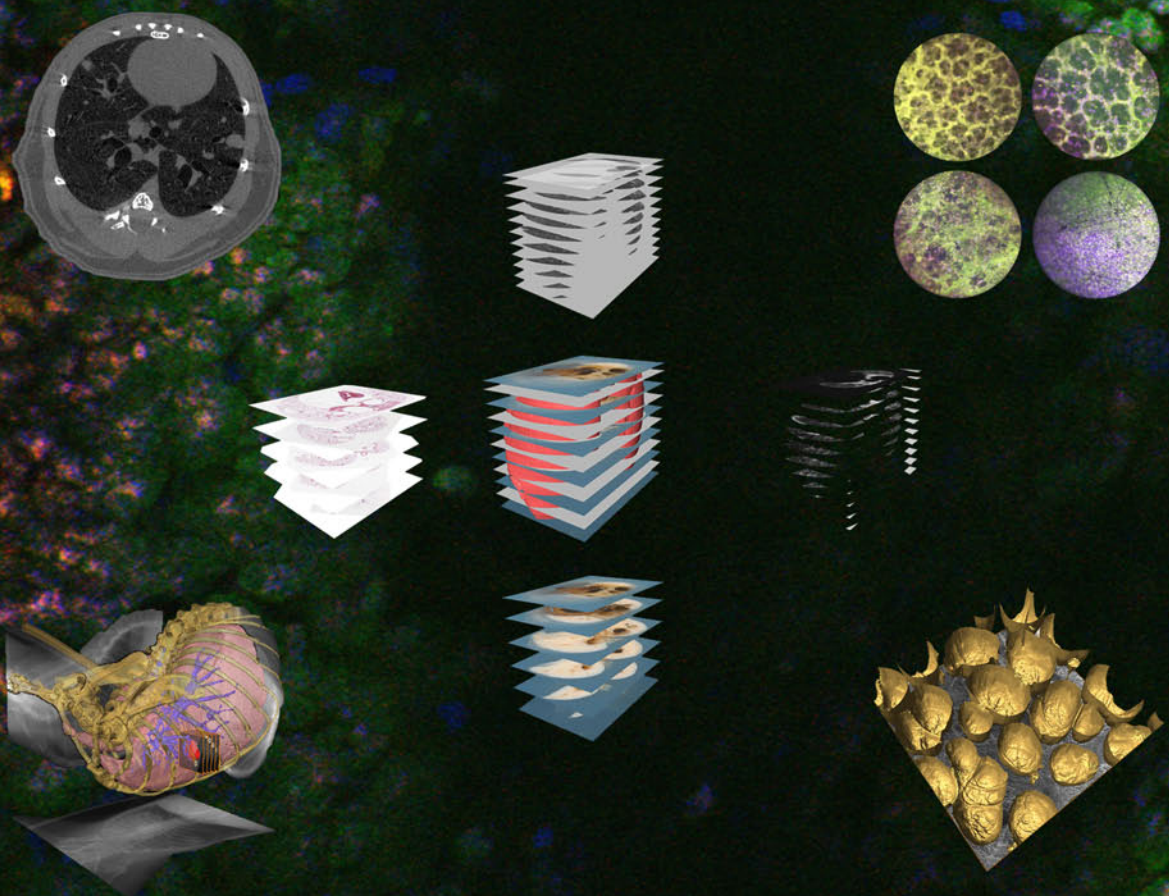
# Pre-Clinical Multi-Modal Imaging for Assessment of Pulmonary Structure, Function and Pathology



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# Pre-Clinical Multi-Modal Imaging for Assessment of Pulmonary Structure, Function and Pathology

by

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# Contents

# Abstract

In this thesis, we describe several imaging techniques specifically designed and developed for the assessment of pulmonary structure, function and pathology. We then describe the application of this technology within appropriate biological systems, including the identification, tracking and assessment of lung tumors in a mouse model of lung cancer.

The design and development of a Large Image Microscope Array (LIMA), an integrated whole organ serial sectioning and imaging system, is described with emphasis on whole lung tissue. This system provides a means for acquiring 3D pathology of fixed whole lung specimens with no infiltrative embedment medium using a purpose-built vibratome and imaging system. This system enables spatial correspondence between histology and non-invasive imaging modalities such as Computed Tomography (CT), Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET), providing precise correlation of the underlying "ground truth" pathology back to the *in vivo* imaging data. The LIMA system is evaluated using fixed lung specimens from sheep and mice, resulting in large, high-quality pathology datasets that are accurately registered to their respective CT and H&E histology.

The implementation of an *in vivo* micro-CT imaging system in the context of pulmonary imaging is described. Several techniques are initially developed to reduce artifacts commonly associated with commercial micro-CT systems, including geometric gantry calibration, ring artifact reduction and beam hardening correction. A computer controlled Intermittent Iso-pressure Breath Hold (IIBH) ventilation system is then developed for reduction of respiratory motion artifacts in live, breathing mice. A study validating the repeatability of extracting valuable pulmonary metrics using this technique against standard respiratory gating techniques is then presented.

The development of an *ex vivo* laser scanning confocal microscopy (LSCM) and an *in vivo* catheter based confocal microscopy (CBCM) pulmonary imaging technique is described. Direct high-resolution imaging of sub-pleural alveoli is presented and

an alveolar mechanic study is undertaken. Through direct quantitative assessment of alveoli during inflation and deflation, recruitment and de-recruitment of alveoli is quantitatively measured. Based on the empirical data obtained in this study, a new theory on alveolar mechanics is proposed.

Finally, a longitudinal mouse lung cancer study utilizing the imaging techniques described and developed throughout this thesis is presented. Lung tumors are identified, tracked and analyzed over a 6-month period using a combination of micro-CT, micro-PET, micro-MRI, LSCM, CBCM, LIMA and H&E histology imaging. The growth rate of individual tumors is measured using the micro-CT data and traced back to the histology using the LIMA system. A significant difference in tumor growth rates within mice is observed, including slow growing, regressive, disappearing and aggressive tumors, while no difference between the phenotype of tumors was found from the H&E histology. Micro-PET and micro-MRI imaging was conducted at the 6-month time point and revealed the limitation of these systems for detection of small lesions (<2mm) in this mouse model of lung cancer. The CBCM imaging provided the first high-resolution live pathology of this mouse model of lung cancer and revealed distinct differences between normal, suspicious and tumor regions. In addition, a difference was found between control A/J mice parenchyma and Urethane A/J mice ‘normal’ parenchyma, suggesting a “field effect” as a result of the Urethane administration and/or tumor burden. In conclusion, a comprehensive murine lung cancer imaging study was undertaken, and new information regarding the progression of tumors over time has been revealed.

# Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Eman Namati

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# Publications

## International Journal Articles (in chronological order).

[1] **Namati, E.,** Chon, D., Thiesse, J., Hoffman, E. A., de Ryk, J., Ross, A., and McLennan, G., "In vivo micro-CT lung imaging via a computer-controlled intermittent iso-pressure breath hold (IIBH) technique," Phys Med Biol, vol. 51, pp. 6061-75, 2006

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# List of Symbols and Abbreviations

$\nu$ ..... Frequency

$\lambda$ ..... Wavelength

18F-FDG ..... Fluedeoxyglucose F18

18F-FLT ..... Fluorothymidine F18

A/D ..... Analog-to-Digital

AOTF ..... Acousto-Optical Tunable Filter

BASC ..... Bronchio-Alveolar Stem Cell

$c$ ..... Speed

CBCM ..... Catheter Based Confocal Microscopy

CCD ..... Charge Coupled Device

cGy ..... Centi-Gray

CT ..... Computed Tomography

DPSS ..... Double Pumped Solid State

dsDNA ..... Double Stranded DNA

$E$ ..... Energy

EM ..... Electro Magnetic

eV ..... electron Volt

FFT ..... Fast Fourier Transform

FOV ..... Field of View

GFP ..... Green Fluorescent Protein

GRE ..... Gradient-Recalled Echo

GRIN ..... Gradient-Index

HU ..... Hounsfield Unit

IFFT ..... Inverse Fast Fourier Transform

IIBH ..... Intermittent Iso-pressure Breath hold

IM ..... Intra Muscular

IP ..... Intra Peritoneal

IV ..... Intra Venous

LE ..... Late Expiratory

LI ..... Late Inspiratory

LIMA ..... Large Image Microscope Array

LSCM ..... Laser Scanning Confocal Microscopy

MCL ..... Mean Chord Length
MCLa ..... Mean Chord Length of Airspace
MCLw ..... Mean Chord Length of Wall
MEMS ..... Micro Electro Mechanical System
micro-CT ..... micro-Computed Tomography
MRI ..... Magnetic Resonance Imaging
MTF ..... Modular Transfer Function
NSCLC ..... Non-Small-Cell Lung Cancer
PBS ..... Phosphate Buffer Saline
PID ..... Proportional Integrative Derivative
PMT ..... Photo Multiplier Tube
RECIST ..... Response Evaluation Criteria in Solid Tumors
rtTA ..... Reverse Tetracycline Transactivator
SCLC ..... Small-Cell Lung Cancer
SNR ..... Signal to Noise Ratio
ssDNA ..... Single Stranded DNA
TTL ..... Transistor-Transistor Logic
WHO ..... World Health Organization