

*Faculty of Engineering*  
*Faculty of Engineering Papers*

---

*The University of Auckland*

*Year 2005*

---

High Resolution 3D Imaging of Lung  
Tissue using Structured Light Microscopy

Yme Kvistedal\*

Peter Hunter<sup>‡</sup>

Merryn Tawhai<sup>†</sup>

Poul Nielsen\*\*

\*University of Auckland,

<sup>†</sup>University of Auckland, m.tawhai@auckland.ac.nz

<sup>‡</sup>University of Auckland, p.hunter@auckland.ac.nz

\*\*University of Auckland, p.nielsen@auckland.ac.nz

This paper is posted at ResearchSpace@Auckland.

<http://researchspace.auckland.ac.nz/engpapers/19>

# High Resolution 3D Imaging of Lung Tissue using Structured Light Microscopy

Y. A. Kvistedal, M. Tawhai, P. Hunter, P. M. F. Nielsen  
Bioengineering Institute, University of Auckland, Auckland, New Zealand

**Abstract**—A 3D reconstruction microscope has been built in order to investigate the structural details of the airway tree and the vasculature of mouse lungs. The objective is to create an anatomically correct finite element model of a mouse lung in order to validate results from simulations obtained using an existing model of the human lung. The 3D reconstruction microscope consists of a fully automated scanning stage, a vibratome and a structured light optical microscope. Structured light microscopy is a new approach to optical sectioning of tissue and offers several advantages over confocal microscopy.

## I. INTRODUCTION

The development of mathematical models of the human lung (Fig 1) has been a long-standing research interest at the Bioengineering Institute, University of Auckland [1-3]. These models have been designed to perform simulations of pulmonary air flow and blood circulation. This research has been instrumental in a global effort to gain insight into pulmonary function and respiratory disorders. It is important to verify the results obtained using the virtual human lung. A 3D reconstruction microscope has been built to obtain information from studies of mouse lungs. The system is designed to perform high-resolution imaging of the tissue, from the whole organ down to capillary level. This will enable us to study the structural details of lung tissue while simultaneously obtaining physiological relevant measurements. We are interested in distinguishing between arteries and veins, identifying how far particles of different size can penetrate into the airways, and identifying the protein distribution within the tissue.

Conventional non-invasive 3D imaging techniques, such as MRI and CT, does not offer the resolution required for imaging the detailed structure of lung tissue. Our approach is therefore to combine vibratome sectioning with a microscope technique capable of optical sectioning, for high resolution imaging in all three dimensions. Structured Light Microscopy [4-6] is a relatively new technique for optical sectioning. It offers a simple and inexpensive alternative to confocal microscopy, and has the advantage that it can be used with a white light source as well as a laser. The main reason for choosing the structured light approach in the 3D reconstruction microscope is that it offered a major improvement in the overall throughput of the system.

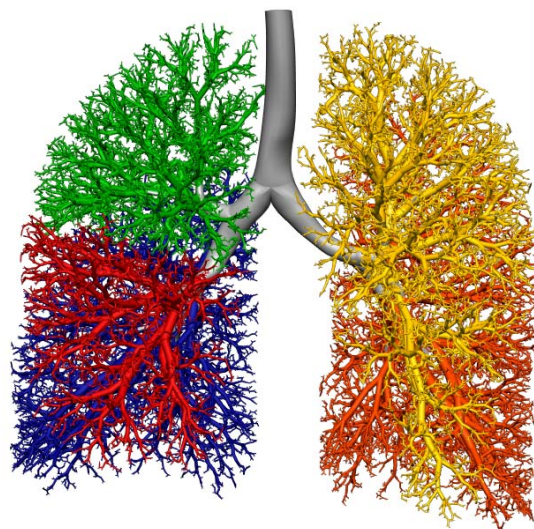


Fig 1: An anatomically correct finite element model of the airway tree of the human lung

## II. METHODOLOGY

The 3D reconstruction microscope (Fig 2) has been build in order to investigate the structure of medium sized tissue samples, such as mouse lungs. The system consists of three parts: an automated scanning stage, a vibratome and an optical microscope. Three high precision linear motor stages (*Daedal*) are configured as a microscope scanning stage with three degrees of freedom. These have a resolution of 0.1 $\mu$ m and a travel range allowing studies of tissue blocks up to 50 x 50 x 50 mm. The motor stages are computer controlled using a multi axis motion control card (*National Instruments*). The scanning stage is used to position the sample with respect to both the optical microscope as well as the vibratome. By using the stages to move the sample while it is being cut with the vibratome, very accurate control the sectioning thickness as well as the feed rate of the cutting is obtained. The vibratome is powered by a solenoid triggered through an analogue output signal from a data acquisition card (*National Instruments*), allowing control of both amplitude and frequency of the blade. The position of the blade is continuously monitored using an infrared reflective sensor and the measured amplitude is used as a feedback variable when cutting the tissue. The rake angle of the blade can be manually adjusted.

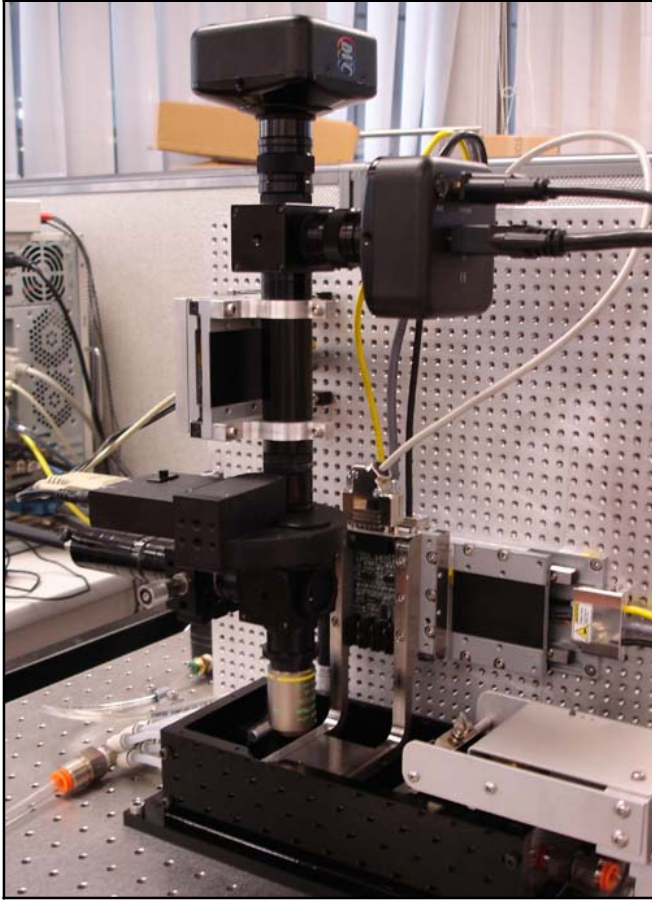


Fig 2: The 3D Reconstruction Microscope.

The surface of the newly exposed tissue is scanned using the optical microscope. The linear motor stages are used to move the tissue with respect to the objective, and a large area scan is composed from up to as many as several thousand images. The optical microscope is custom built using c-mount components. It uses infinity corrected objectives (*Nikon*) and is equipped with both axial and oblique illumination. Two output ports are equipped with highly sensitive CCD cameras (*DVC*). One of which is used for standard brightfield imaging, while the second is used for specific imaging modalities (i.e. spectroscopic imaging in combination with a c-mount spectrometer (*Specim*), or polarized light microscopy). The two cameras use a cameralink interface and are controlled by separate frame grabber cards (*National Instruments*). The optical microscope is designed to support fluorescence imaging and is equipped with motorised filter wheels (*ThorLab*) for both the excitation and barrier filters. The barrier filter wheel can also be equipped with RGB filters for true colour imaging.

All of the individual components of the 3D reconstruction microscope is controlled via a single computer, using software developed with LabView

(*National Instruments*). This enables fully automated studies to be performed over extended periods of time.

A common problem when imaging a block of tissue with a high magnification microscope is that light will be reflected from structures outside the focal plane. This results in noise within the images and effectively decreases the z-resolution of the microscope. The standard technique for avoiding this problem is to use a confocal microscope. In order to increase the z-resolution, by removing out of focus information from the images, the 3D reconstruction microscope has been designed for structured light microscopy. This is a relatively new technique that has the potential to replace confocal microscopy. A digital mirror device (*DMD*) (*Texas Instruments*) is used to illuminate the sample with binary patterns through the objective. As this light hits the sample at an angle, given by the numerical aperture of the objective, the dark parts of the pattern will be restricted along the z-axis as well as within the image plane. Two images with inverted illumination patterns will thus be equally illuminated outside the focal plane, and the out of focus information removed by digitally subtracting the two images. The major advantage of using structured light microscopy over confocal microscope in the 3D reconstruction microscope is that it offers a major decrease in acquisition time. This is important, as a single sample can easily exceed hundreds of thousand images in a full 3D scan.

The tissue is fixed in formalin and embedded in agar before imaging. To improve contrast the agar is stained black using confectionery colour. The tissue is imaged and sectioned while immersed in a bath containing an oil solution. This both help lubricate the vibratome blade during cutting as well as stopping the tissue from drying. A peristaltic pump (*Ismatec*) continuously circulates the solution through a filter, removing cutting debris. Water dipping objectives are used for higher magnifications (10x and above), while a coverslip on the surface of the bath is used when imaging with smaller dry objectives. In order to control the temperature of the tissue and to freeze the sample, the scanning stage has been equipped with a thermoelectric device (*Marlow*).

### III. DISCUSSION

There are several challenges with 3D microscopy. When imaging a large sample with a high magnification objective, optimising the throughput and data handling of the system become very important. As an example, imaging a volume of 50 x 50 x 50 mm using a 10x objective requires the composite surface image to be built up of more than 9000 individual images. When this scan is repeated at 10 $\mu$ m intervals along the z-axis the acquired number of images becomes a staggering 45 million. The structured light

microscopy has offered a major increase in throughput compared to similar systems using confocal microscopy [7, 8]. Acquiring a single image takes approximately one second, including time for moving the stage. Therefore scanning a full sample would require about 520 days. A second issue is the amount of data acquired by the microscope. When using 8bit PNG compression each image is about 1MB in size. The above example would require 45TB of storage capacity. Both the acquisition time and storage space introduce practical limitations to the system, and thus there is a trade off between sample size and image resolution.

An issue also related to the throughput and accuracy of the system, is the stability of the tissue. Any change within the tissue during the imaging process will result in misalignments between the image planes. The ability to control the humidity, temperature and osmolality of the tissue and the bath solution has, to a large extent, been successful in maintaining tissue stability. However due to the extensive time requirements we have found that it is important to either fix the tissue with formalin or to freeze the sample throughout the imaging process.

To improve the accuracy of the alignment between the image planes, it is also very important to minimise any deformations in the tissue resulting from the vibratome sectioning. By mounting the tissue block on a force platform we can measure the magnitude and direction of the forces the blade imposes on the tissue. By adjusting the cutting parameters accordingly we can minimize these deformations and improve the overall accuracy of the system.

## REFERENCES

1. Tawhai, M.H. and P.J. Hunter, *Modeling water vapor and heat transfer in the normal and the intubated airways*. Annals of Biomedical Engineering, 2004. **32**(4): p. 609-22.
2. Tawhai, M.H. and K.S. Burrowes, *Developing integrative computational models of pulmonary structure*. Anatomical Record. New Anatomist, 2003. **275**(1): p. 207-18.
3. Burrowes, K.S., M.H. Tawhai, and P.J. Hunter, *Modeling RBC and neutrophil distribution through an anatomically based pulmonary capillary network*. Annals of Biomedical Engineering, 2004. **32**(4): p. 585-95.
4. Fukano, T. and A. Miyawaki, *Whole-Field Fluorescence Microscope with Digital Micromirror Device: Imaging of Biological Samples*. Applied Optics, 2003. **42**(19): p. 4119-4124.
5. Mitic, J., T. Anhut, M. Meier, M. Ducros, A. Serov, and T. Lasser, *Optical sectioning in wide-field microscopy obtained by dynamic structured light illumination and detection based on a smart pixel detector array*. Optics Letters, 2003. **28**(9): p. 698-700.
6. Neil, M.A.A., R. Juskaitis, and T. Wilson, *Method of obtaining optical sectioning by using structured light in a conventional microscope*. Optics Letters, 1997. **22**(24): p. 1905-07.
7. Young, A.A., I.J. LeGrice, M.A. Young, and B.H. Smaill, *Extended confocal microscopy of myocardial laminae and collagen network*. Journal of Microscopy, 1998. **192**(Pt 2): p. 139-50.
8. Hanley, P.J., A.A. Young, I.J. LeGrice, S.G. Edgar, and D.S. Loiselle, *3-Dimensional configuration of perimysial collagen fibres in rat cardiac muscle at resting and extended sarcomere lengths*. Journal of Physiology, 1999. **517**(Pt 3): p. 831-7.