Bronchoscopic imaging of pulmonary mucosal vasculature responses to inflammatory mediators

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Abstract. In current clinical practice, the initial stage in the detection of pulmonary airway disease is the clinician's interpretation of a bronchoscopy procedure. Due to the nature of this form of detection, diseases are often well advanced upon discovery. This paper proposes a new method for examining the pulmonary airway microcirculatory system via fluorescein imaging techniques similar to those used extensively in ophthalmology. Through the use of a specialized bronchoscopy imaging system we are able to successfully show that fluorescein emission detected in the rabbit trachea exhibits a clear response to inflammatory mediators and thus to changing local vascularity. The clinical implications of this technique are extensive including most importantly the potential use as an evaluation tool for pulmonary disease evaluation and likely other endoscopically accessible organs.
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1 Introduction

Qualitative optical analysis of the airway mucosa during a bronchoscopic procedure is currently the initial step in the identification of pulmonary airway disease. Due to the inherent insensitivity of this detection method the critical initial stages of the disease often remain undetected and correct diagnosis only occurs once the disease is in advanced stages. Alterations to airway mucosa microvasculature frequently occur in response to the development of pulmonary airway diseases such as lung cancer, inflammatory bronchitis, or cystic fibrosis. As such, investigating the subepithelial vasculature is crucial to enhancing our understanding of airway responses to changing mucosa structure and composition.[1]

To date, most investigators have focused on the larger vessels of the pulmonary circulation due to the difficulty associated with measuring blood flow in the microcirculatory system. Shibuya et al.[2] have examined the subepithelial microvascular using a high magnification bronchovideoscope. This imaging method has an extremely small field of view and is problematic when attempting to quantitatively measure vascular effects. The aim of our research is to provide an effective and relatively noninvasive means of observing large field alterations in tracheal mucosal blood flow in response to various inflammatory mediators. It is our continuing view that macro-optical techniques, such as the one described in this paper, will provide smaller relevant regions of interest for the micro-optical techniques such as those described by Shibuya et al.[2] We see the fluorescein approach therefore as being complimentary to that of Shibuya et al.

Fluorescein angiography is commonly used to qualitatively evaluate the circulation of the retina in order to detect and diagnose diseases including diabetes.[3,4] The process of fluorescein angiography involves injecting a bolus of fluorescein into the patient's circulatory system and observing the time based response in the retina.[5] Fluorescein is a highly fluorescent chemical compound that upon excitation with a blue light source between 465 and 490 nm spontaneously fluoresces at the yellow-green wavelengths of 520–530 nm.[6] A large portion of the fluorescein injected into the blood stream binds to

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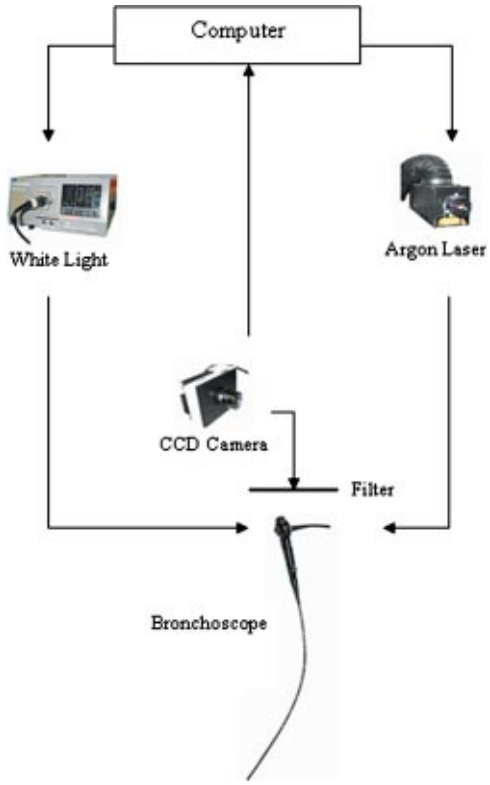

Fig. 1 Flow diagram of the fluorescent imaging system for use in the detection of green fluorescent protein in the pulmonary airways. The bronchoscope is used to visualize the airways in conjunction with the white light and argon laser. The CCD camera captures images as seen by the bronchoscope with the laser light removed. The personal computer (PC) controls the entire system operation.

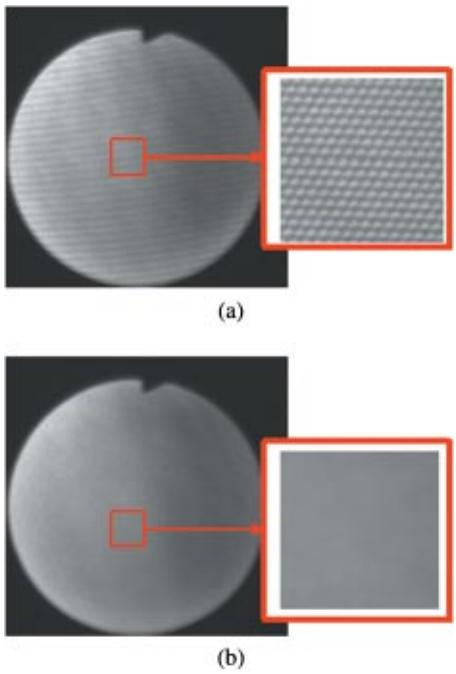

Fig. 2 (a) White light image captured using the fluorescent imaging system highlighting the underlying fiber distortion and (b) the image postapplication of the Fourier domain filtering.

serum protein, however it is the unbound fluorescein molecules that are responsible for the observed light emission.[6] Fluorescein remains in the body for up to 36 h before being completely metabolized by the kidneys.[7]

In addition to the use of fluorescein in ophthalmology applications we believe that fluorescein has a potential use in determining changes in airway mucosa vasculature. By monitoring the fluorescent emission in the airway tree we hypothesize that regions of airway mucosa corresponding to high or low intensity fluorescent emission will correlate with high or low tissue vascularity.

2 Methods

Detection of the fluorescein emission in the pulmonary airways requires the use of a specialized imaging system capable of providing the necessary excitation and also accurately detecting the fluorescein emission. We have previously constructed an imaging system of this type (Fig. 1) designed to enable the detection of green fluorescent protein in the airway epithelia used in conjunction with gene therapy research.[8] This fluorescent imaging system has an exposure time of 0.07 s ensuring image acquisition at a sufficient speed so as to avoid any potential adverse affects from cardiac motion that may otherwise be problematic in the left main bronchus.

As is the case of standard clinical practice, a bronchoscope (bronchofiberscope, BF type 1T40, Olympus, Melville, NY) used in conjunction with a white light illumination source (CLE-10, Olympus) is required to examine the airways. Excitation is provided via an 800 μm fiber optic cable (Sharplan Laser Co., Allendale, NJ) delivering the output from a 488 nm internal mirror argon ion laser (IMA-102-010-BOS, Melles Griot Laser Group, Carlsbad, CA) through the distal end of the bronchoscope. The fiber optic cable terminates in a hemispherical diffusing tip to uniformly distribute the excitation light onto the airway walls. During system operation a foot pedal is engaged to switch between these excitation sources and simultaneously send an output signal via a microcontroller (Parallax Basic Stamp II, Parallax Inc., Rocklin, CA) to the operating system to signal a change in image acquisition mode.

In order to differentiate between the emitted fluorescein and the excitation source a 488 nm band-reject filter is positioned over the eyepiece at the proximal end of the bronchoscope. The role of the filter is to occlude the laser light ensuring that all detected illumination is fluorescein emission. The entire system is coupled to a Photometrics SysSys, high performance charged coupled device (CCD) camera (Photometric Ltd., Tuscon, AZ) facilitating computer controlled acquisition and analysis of digital images of the fluorescein emission.

The use of fiber optic bronchoscopes in the evaluation of the airways continues to be used extensively throughout the world. Images captured by the fiber optic bronchoscope and CCD camera assembly display an underlying grid pattern located throughout the bronchoscope field of view [Fig. 2(a)]. This grid pattern or fingerprint is due to the high resolution CCD camera capturing the spaces between the individual fibers of the bronchoscope. In an attempt to remove this perceived distortion a filtering process was applied. In fingerprint

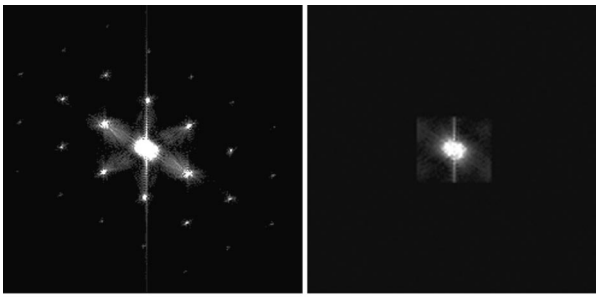

Fig. 3 (a) The Fourier transform magnitude of the image depicted in Fig. 2(a) clearly showing the periodic noise of the underlying grid pattern. The periodic noise from the bronchoscope fibers corresponds with the bright regions surrounding the center mass. (b) The resulting low pass filtered image in the frequency domain.

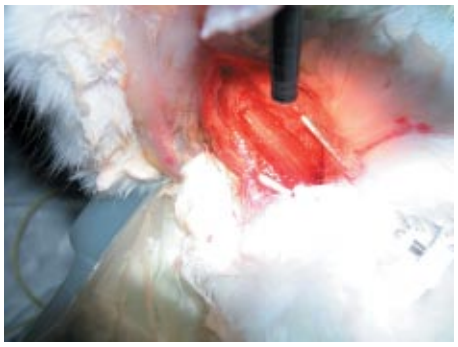

Fig. 4 Exposed trachea of a New Zealand white rabbit.

enhancement techniques, filters are designed to transmit peak spectra corresponding to ridge frequencies and directions and to attenuate the remaining signal thus enhancing the fingerprint pattern.[9] Radial bandpass filters are commonly used for this purpose. Each filter is tailored to match the patterns of the individual fingerprints by observing the two-dimensional (2-D) Fourier power sprectra. We however wish to attenuate the spectra corresponding to the fiber grid, and transmit all remaining spectra. The underlying grid produces a periodic noise signal which can be viewed in the frequency domain [Fig. 3(a)]. Similar problems have been encountered with microendoscopes, where the noise can be reduced with band reject filters.[10] Due to the observed Fourier spectrum of our system, such methods are not sufficient. Because of the large number of peaks corresponding to noise from the fiberoptic scope, a box low-pass filter was used to attenuate these high frequency noise components and effectively greatly reduce the contrast between each of the individual fibers of the bronchoscope. In order to determine the cutoff frequencies for the low-pass filter, manual matching of the filter and the Fourier spectra of an image showing the distortion, was performed. The frequency spectra corresponding to the underlying grid pattern does not vary between images captured with the bronchoscope system, and thus these chosen cutoff frequencies are applicable to all images captured. An inverse Fourier transform is then applied to the filtered frequency domain image to produce the resulting image [Fig. 2(b)].

Low-pass filtering in the frequency domain essentially decreases the sharpness of an image, thus careful consideration in cutoff frequency determination is required. If the chosen cutoff frequency is too low, significant image information will be lost. In contrast, should the cutoff frequency be too high, insufficient grid removal will result.

Region of interest segmentation is required when determining the frequency response in specific sections of the exposed tissue. Once the bronchoscope imaging system and subject have been positioned and fixed in place, a white light image is captured with the imaging system. Images captured with laser light excitation are predominately dark and show very little scene detail other than the fluorescein emmision, thus the white light image is required to select the regions of interest. In the case where the regions of interest are clearly distinguishable in the white light image, they can be extracted using image processing techniques. A combination of image thresholding, morphological closing, region growing, and labeling image processing techniques are used to create a binary image of the extracted regions. Region growing requires an initial seed point, within the boundaries of the desired area of interest, at which to start the region of interest determination. The seed pixels for the region growing process are manually chosen to correspond to each region in the image. This binary mask is then used to determine the fluorescein response in each of the captured images. The mean fluorescein emmision is determined for each of the regions based on the pixel intensities and the region areas.

3 Experimental Methods

The tracheal mucosal blood flow accounts for nearly 85% of the total tracheal blood flow and has been shown to vary considerably in rabbits between 8.9 and 22.7 mL min^{-1} per 100 g tissue.[11] This inconsistency in basal tracheal microvasculature was not found in intrarabbit studies, but rather varied within the rabbit population. No similar such data was found for human studies presumably due to the required invasiveness of the measurement techniques.[1] Despite the large mucosal blood flow measurements the majority of research has generally focused on the larger blood vessels highlighting the need for further research to be conducted in this area.

In order to confirm our initial hypothesis that an increase in vascularity of the airway mucosa produces a corresponding increase in the detectable fluorescein emission, we designed an experiment involving one New Zealand white rabbit. The experiment was approved by, and carried out in accordance with the guidelines set by the University of Iowa Institutional Animal Care and Use Committee. The rabbit was anesthetized (35 mg kg^{-1} of ketamine and 5 mg kg^{-1} of xylazine) and underwent a surgical procedure to expose a 5 cm length region of the posterior inner tracheal wall (Fig. 4). The bronchoscope tip was positioned 2 cm above this tracheal window, and clamped in place to allow for continuous monitoring without moving the bronchoscope or the animal.

Alterations in subepithelial vascularity were achieved using various inflammatory mediators including the vasodilators bradykinin (20 μM) and histamine (10 mM), the vasoconstrictor cocaine (0.1%) as well as a saline control. Each of the inflammatory mediators were applied in 10 μL measures to one of four 5 mm diameter circular filter papers. The four

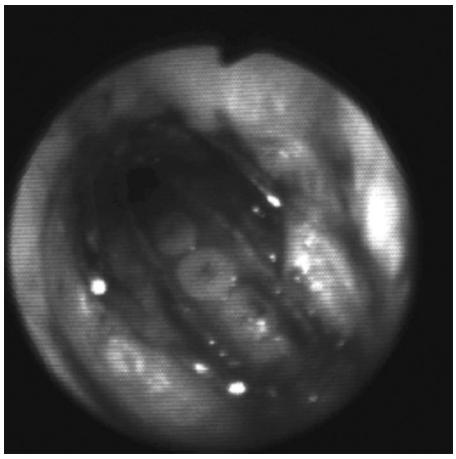

Fig. 5 Exposed trachea of the New Zealand white rabbit showing the placement of the filter papers each prepared with an inflammatory mediator. The image was captured using the bronchoscope imaging system under white light illumination.

patches were all consequently applied to the previously exposed rabbit tracheal tissue in for a period of 1 min (Fig. 5).

Immediately following removal of the inflammatory mediator prepared filter papers the rabbit was injected through an ear vein with a 7.7 mg kg^{-1} dose of fluorescein at which time continuous image acquisition, with excitation from the laser source, of the exposed trachea began. Detection of the time course of the fluorescein continued for a period of 90 min before the rabbit was sacrificed due to anesthetic time limitations.

4 Results

Upon termination of the fluorescein response monitoring in the rabbit trachea, image processing techniques, including region of interest segmentation, were applied to the image series. The regions corresponding to the inflammatory mediator exposed epithelial tissue were extracted and numerical measurements made. Figure 6 shows the initial time based mean pixel values of each of the extracted regions. These results clearly show that the detected fluorescence varies amongst each region.

As can be noted from Fig. 6, approximately 4 s lapsed before the initial sign of the fluorescein circulating in the blood was detected. The detected fluorescein appears to reach a maximal peak at 11 s postinjection of fluorescein after which the levels appear to approach a reasonably steady state.

5 Discussion

Images of the fluorescein-induced fluorescence were successfully detected exhibiting no signs of image blurring due to motion artifacts including from the cardiac motion, and an image acquisition time of 0.07 s appears short enough to extend these observation into the human situation. Histamine and bradykinin are both potent vasodilators therefore suggesting that the blood volume in the region of the tracheal mucosa that has been exposed to these mediators would increase. A corresponding increase in the detected fluorescein emission is expected. Conversely in the case of mucosa having been exposed to a vasoconstrictor such as cocaine we expect the opposite to be true with the blood volume and hence the fluorescein emission decreasing. As Fig. 6 shows, these intuitive results were confirmed with our experimental observations.

Note the small decrease in the fluorescein emission detected for the cocaine-exposed tissue compared with that of the saline control, suggesting the possibility that in this particular animal the posterior tracheal mucosal blood flow was already very low, and was further influenced by the cocaine, although not greatly so. The basal mucosal blood flow was, however, detectable as a fluorescein signal.

As previously mentioned, fluorescein remains in the blood for a period of up to 36 h before being completely metabo-

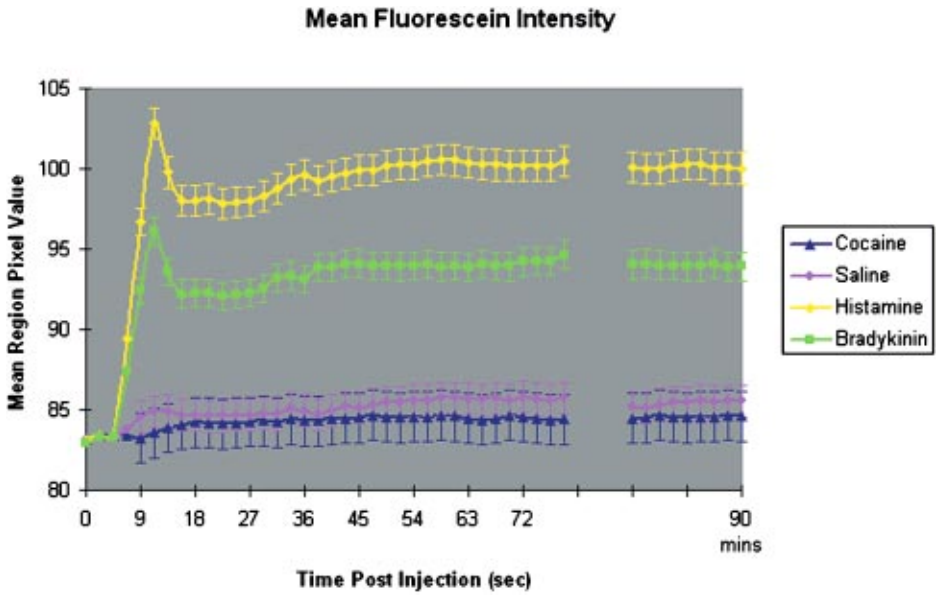

Fig. 6 Detected mean fluorescein emission, presented in mean pixel value with pixel error bars, for each of the four tissue regions exposed to the inflammatory agents.

lized by the kidneys. This long life span made it difficult for us to monitor the entire time course of the fluorescein in the rabbit trachea due to anesthetic limitations and thus the experiment was concluded after approximately 90 min.

6 Conclusions

The aim of this research as previously stated was to provide an effective and relatively noninvasive means of observing normal and abnormal changes in tracheal mucosal blood flow. In order to do this we hypothesized that through detection of administered fluorescein in the trachea we would be able to detect alterations in the mucosal blood flow. Our results demonstrate that we have been able to effectively support this hypothesis using known vasodilator and vasoconstrictor agents in the trachea. Human investigations are currently in progress; if important new knowledge can be acquired using this technique then the low cost of fluorescein and its extremely low side effects profile may be important factors in its more wide-spread use for these purposes.

The clinical significance of this research is to potentially develop a pulmonary airway evaluation system to detect possible disease. It is believed that in the early stages of lung cancer development, and other pulmonary diseases, a change in mucosal vascularity occurs due to the changing tissue structure and composition and hence nutrient requirements. Through clinical evaluation of the patient airway using the developed fluorescent bronchial imaging system in conjunction with fluorescein administered intravenously we believe that successful detection of abnormal pulmonary mucosal vascularity is possible. The developed imaging system used in conjunction with virtual bronchoscopy and endoscope matching software[12] will also facilitate the tracking of fixed airway locations over time. Detection of such changes prior to the disease reaching advanced stages, when diagnosis commonly occurs, would potentially increase the effectiveness of current patient treatments as well as provide a greater understanding of the airway microvascular system.

Acknowledgments

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