Dynamic imaging of *in vitro* human airway epithelium using optical coherence tomography

Amy L. Oldenburg,[1,2] Raghav K. Chhetri,[1] Brian M. Button,[3] David B. Hill,[3] and Richard C. Boucher[3]
[1]Department of Physics and Astronomy, [2]Biomedical Research Imaging Center, [3]Cystic Fibrosis Center,
The University of North Carolina at Chapel Hill, USA
aold@physics.unc.edu

Abstract: Ultrahigh-resolution optical coherence tomography (OCT) is employed to depth-resolve mucociliary transport on human bronchiepithelial cell cultures. This has relevance for monitoring airway mucus in lung diseases such as cystic fibrosis and COPD.
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1. Introduction

During respiration, the human body inhales airborne pathogens and irritants that are deposited onto the surface of the airways. The airway surface liquid (ASL) protects against these pathogens. The ASL is comprised of an outermost mucus layer and an inner periciliary layer (PCL) in which cilia beat to clear mucus from the airways. In order to prevent infection, the mucus layer must transport the pathogenic materials from the lungs via mucociliary transport or cough clearance. Diseases such as cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD) are characterized by a loss of mucociliary transport, leading to chronic lung infection. Because mucus in these diseases is often abnormally thick and turbid, depth ranging techniques such as optical coherence tomography (OCT) are well-suited to monitor and depth-resolve mucociliary activity. While clinical OCT systems for imaging in human airways are of increasing interest,[1-4] here we report a pre-clinical study of the efficacy of ultrahigh-resolution (UHR) OCT for characterizing mucociliary transport in an *in vitro* model employing human bronchiepithelial (hBE) cell cultures. These cultures are grown at an air-liquid interface and coordinate into an artificial airway epithelium that exhibits mucociliary transport.

2. Methods

2.a. hBE cell cultures

Human bronchiepithelial cell cultures were prepared as described in detail previously.[5] Briefly, hBE cells were removed by protease dissociation from bronchi from excess donor tissue according to protocols approved by the UNC Institutional Review Board. Cells were seeded onto the surface of a membrane immersed in growth media. Upon reaching confluence the culture medium is excluded from the apical side, cilia are observed to beat, and over 2-4 weeks mucus is produced and exhibits rotational transport. The cultures typically maintain a 7 μm thick PCL,[6] and facilitate coordinated transport of the mucus layer.[5, 7]

2.b. UHR-OCT system

The ultrahigh resolution OCT system is a spectral-domain system that has been described in detail previously.[8] Briefly, the light source consisted of a femtosecond Ti:Sapphire laser (Griffin, KMLabs, Inc.) at a center wavelength of 810nm and bandwidth of typically 125nm, providing an experimentally obtained axial resolution of 3μm in tissue and a power of 10mW at the sample. A 30mm focal length lens provided a lateral resolution of ~12μm. The OCT system was comprised of a free-space Michelson interferometer and single mode fibers to steer the beam to the custom spectrometer, which was comprised of a CCD line camera operated at 1kHz or 5kHz (Dalsa Pirahna 2). Images were acquired either in M-mode (repetitive axial scans of 1.5mm in depth sampled at 1kHz) or B-mode (1.5 × 1.5 mm in $x \times z$) and were sampled into 1000 × 1024 pixels.

3. Results and Discussion

As shown in Fig. 1, it is possible to image the PCL even through abnormally thick mucus (in this case ≈200μm). The top and bottom of the supporting membrane appears as two strongly backscattering dark bands in the OCT images. The PCL is evident, particularly in M-mode, as a layer exhibiting more rapid light scattering fluctuations. In a single B-mode image the PCL is only weakly scattering, however, movies generated by successive B-mode frames at 2Hz (not shown) elicit the idea of slow mucus transport under which the PCL is rapidly fluctuating.

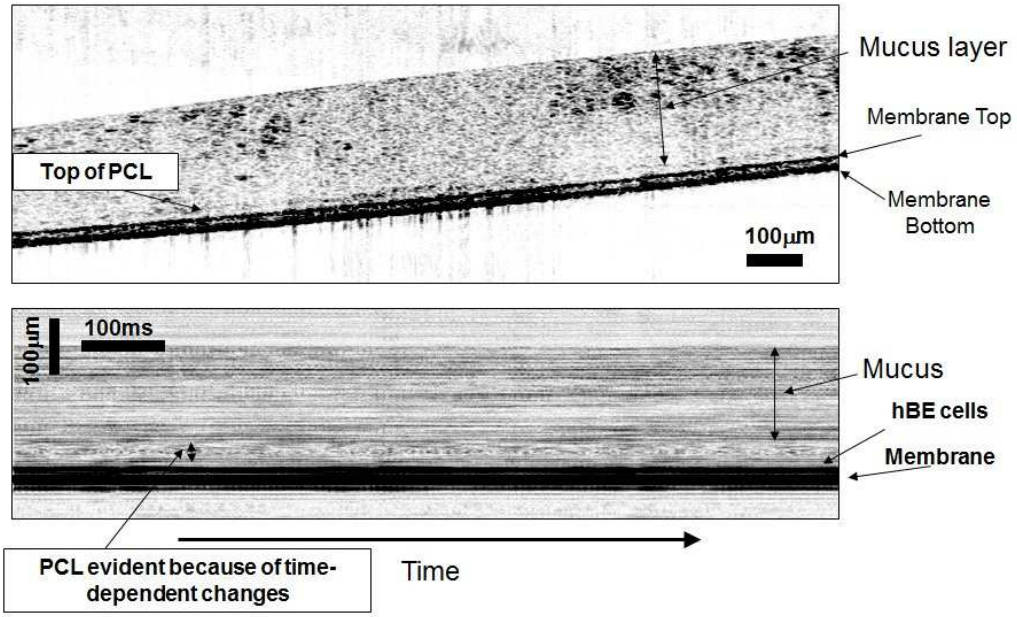

Fig. 1. Top: B-mode OCT showing depth-resolved structure. Bottom: M-mode OCT at 1kHz performed in the center of the top image showing time-resolved motion.

To quantify the depth-dependent dynamics of this model airway epithelium, we processed the M-mode image to estimate the speckle decorrelation rate. This was performed by computing the autocorrelation at each depth after subtraction of the mean value, then averaging the autocorrelations within windows of 30μm for statistical averaging. For each window, the random noise peak at $\tau=0$ was ignored, and the first value ($\tau=\Delta t$) was taken as the peak value. The decay time was then defined as the time to reach 75% of the peak value, since the use of a more standard $1/e$ definition tended to extend into noisy regions of the autocorrelation traces (due to finite temporal sampling).

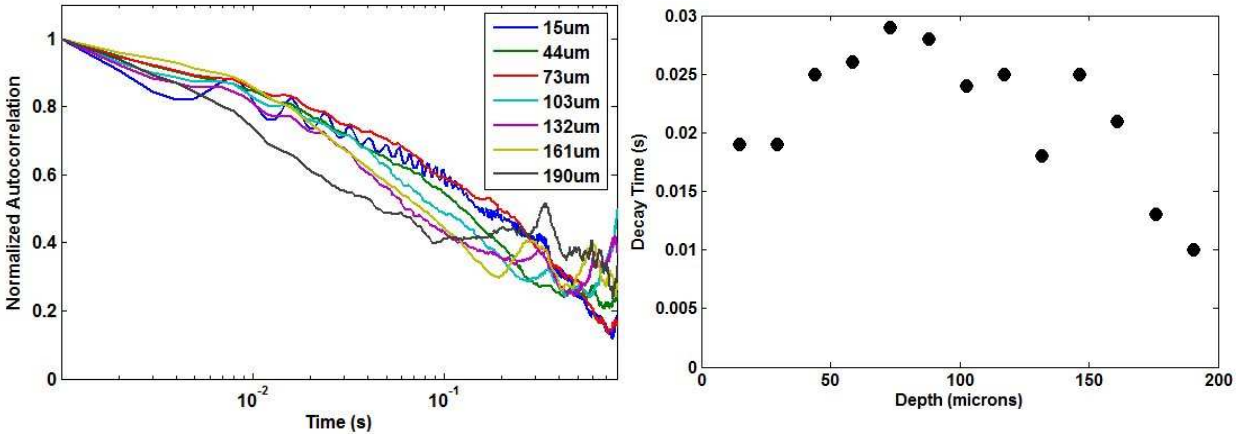

Fig. 2. Left: Temporal autocorrelations of the depth-dependent OCT signal averaged within 30μm windows centered at the depths indicated with respect to the mucus-air interface. Right: Associated decay times (defined as the time to reach 75% of the peak value) versus depth.

The results of these processing steps on the M-mode image of Fig. 1 are illustrated in Fig. 2. The decay time (e.g., the speckle decorrelation time) varies between 18-30ms throughout the bulk of the mucus layer. However, in the region containing the PCL, a sharp drop to 10-13ms is observed, as anticipated based upon visual inspection of Fig. 1. Measuring from Fig. 1, the region of dynamic activity correlated with the position of the PCL appears to be nominally 20μm thick which covers a large fraction of the 30μm windows near the bottom of our region of analysis. Further reduction of our window size to better resolve the PCL might be possible in future work with better

statistical sampling in other dimensions, such as time, or higher resolution. We also note that microscopic analysis of the PCL in these models reveals that it is typically only 7 μm thick[5], suggesting that the dynamic light scattering changes observed in OCT extend over a broader region than the physical extent of the PCL. Clearly, our UHR-OCT system resolution of 3μm is sufficient to resolve this active region associated with the PCL, although a higher resolution micro-optical OCT system has recently been reported which can resolve individual cilia [9].

Conclusions

In summary, we have demonstrated the use of UHR-OCT for monitoring the functional dynamics of mucociliary transport in an abnormally thick *in vitro* model system of the human airway which models disease states such as COPD and CF. A decrease in speckle decorrelation time near the PCL is associated with ciliary activity. In future work, methods to quantify the mucus transport rate can be accomplished by relating decorrelation rate with velocity, or alternately, by speckle tracking via spatial cross-correlations. The methods developed with this *in vitro* system may be translatable to bronchoscopic OCT for clinical applications such as monitoring mucus-thinning therapies aimed at restoring mucociliary transport.

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