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High-speed Imaging of Cilia Beat Frequency using Phase Resolved Spectrally Encoded Interferometric Microscopy

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ABSTRACT

Mucociliary clearance is vital for preventing any foreign substances from entering the upper airway that can later develop into acute and/or chronic respiratory diseases. Therefore, it is essential to further advance our understanding of the mucociliary functions. Our lab has been able to make key developments in imaging cilia, specifically measuring cilia beat frequency, with phase-resolved Doppler optical coherence tomography. In this system, we have further developed the system by incorporating phase-resolved spectrally encoded interferometric microscopy (SIEM) system with an FDML laser with MHz sweep rate to image cilia with higher accuracy and to minimize motion artifacts. In addition, we have designed a compact handheld probe system with a GRIN lens for easier in vivo imaging. The development of this system will allow us to further investigate cilia dynamics and ultimately utilize the system for clinical applications.

Keywords: mucociliary clearance, cilia beat frequency, spectrally encoded interferometric microscopy, phase-resolved, endoscopy

1. INTRODUCTION

Mucociliary clearance is the first line of defense for the upper respiratory track and is heavily dependent of proper cilia dynamics. Cilia, which are elongated, hair-like cells, sways back and forth in a synchronized movement at a fundamental frequency called the cilia beat frequency (CBF) to push the foreign particles out of the respiratory tract. CBF can be affected by numerous factors such as temperature, humidity, and pharmaceutical application. By studying the cilia dynamics, we could further understand and access the efficacy of different treatments. [1]
Our goal is to use spectrally encoded interferometric microscopy (SEIM) to image cilia at high-speed. Phase-resolved SEIM has already been shown to be able to measure CBF via *ex vivo* [2]

2. METHODS

2.1 Phase-Resolved Spectrally Encoded Interferometric Microscopy (PR-SEIM)

SEIM is a technique that is derived from OCT. A grating is added to the system which diffracts the beam. Using the diffracted beam, the system can take high speed *en face* images with the focal plane parallel to the tissue sample instead of perpendicular to the sample. The system set up is shown in figure 1.

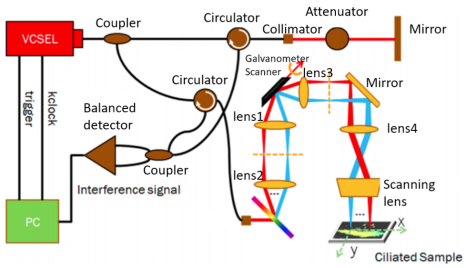

Figure 1: Set up of the *ex vivo* benchtop PR-SEIM system [3]

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2.2 *Ex vivo* Collection

Tissue

This study was approved by the University of California, Irvine Institutional Review Board. Tissues from patients undergoing sinus surgery were used to image. It was placed in saline solution and on a hot plate to ensure that its condition would be maintained. The tissue was then placed on a petri dish to be imaged with the SEIM system *ex vivo.*

Collection

The images were taken with a benchtop setup with an objective lens as the scanning lens. The FOV was 900 µm x 900 µm, and the resolution was 2 µm. Using the phase-resolved algorithm developed by previous lab members, the phase of each image was collected at 100 fps (frames per second).

2.3 *In vivo* Collection

Rabbits were placed under anesthesia and incubated to image the nasal cavity. We developed an endoscopic system to image *in vivo* using GRIN (gradient index) lens as the scanning lens and designed to be handheld. The design of the system is shown in figure 2.

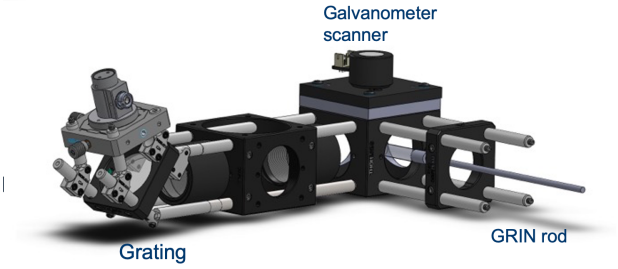


Figure 2: Design of handheld endoscopy system for *in vivo* imaging

3. RESULTS

3.1 *Ex vivo* CBF calculation

Post processing was done to calculate the CBF. Using MATLAB, the mean CBF of the desired ROI was calculated. Due the phenomenon called phase wrapping, calculating the mean CBF of the whole ROI led to inaccurate CBF values. To resolve this, we attempted to image faster to increase the maximum displacement that the system could measure. We increased the frame rate from 100 fps to 400 fps. Although, we were able to see less phase wrapping, it did not significantly change. We were able to overcome this issue by looking at the averaged frequency spectrum instead of the mean CBF. The phase wrapping lead to higher harmonic generation which we used to find the fundamental frequency. In figure 3, there are 3 peaks with the lowest frequency peak being the strongest.

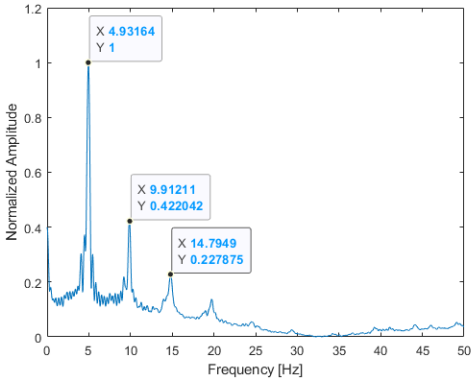
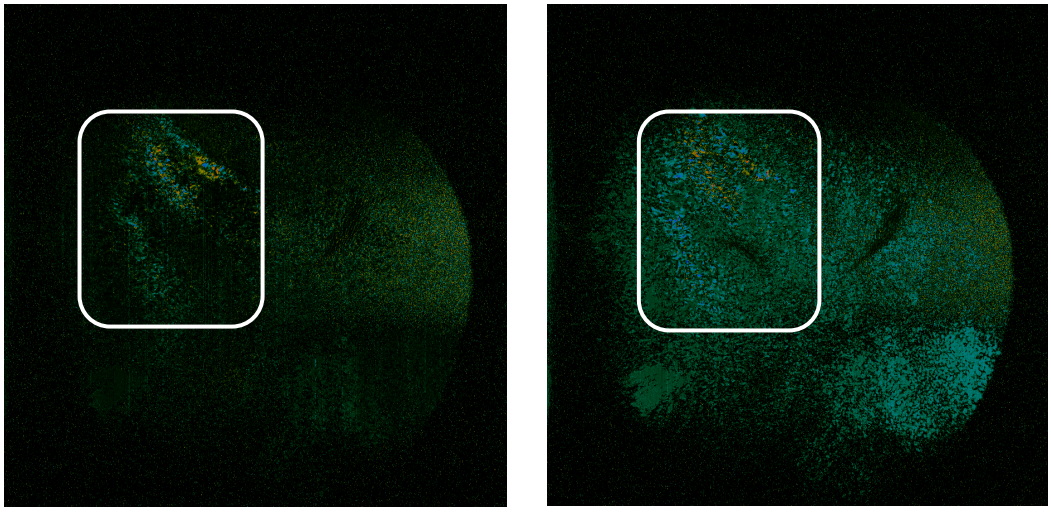


Figure 3: Frequency spectrum of CBF within ROI showing the fundamental frequency, the second harmonic and the third harmonic.

3.2 *In vivo* Images

Phase-resolved SEIM is sensitive to motion artifacts. Although there is bulk motion shown, you can see cilia through the bulk motion where it is brighter consistently as shown in figure 4 (a) and (b). We attempted to calculate the CBF from the images taken, but due to the bulk motion being within the 5 -8 Hz range, we were unable to accurately calculate the CBF. Typical CBF values range from 7-16 Hz for humans.



(a) (b)

Figure 4: Frames of *in vivo* images (a) less bulk motion, (b) with more bulk motion.

4. DISCUSSION

More of the research is now focused on the *in vivo* collection, focusing on the motion compensation as shown in the results. The standard method to calculate CBF is phase contrast microscopy which is can only be use for *ex vivo* images. *In vivo* imaging will allow a deeper understanding of cilia dynamics by studying it in its natural environment.

5. CONCLUSION

Proper cilia dynamics is essential to our respiratory system; therefore, it is vital to study it in more depth. This paper focuses on using phase resolved SEIM to pursue that goal. For *ex vivo* studies, we have confirmed that higher harmonic frequencies could be used for more accurate CBF calculations. We have also shown quantitative results for *in vivo* studies.

6. REFERENCES

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