as the resonance frequency at low stimulus intensities, of ~9 kHz. The CF shifts lower with increasing stimulus intensity, consistent with the cochlear nonlinearities found in larger rodents [1,7]. The vibration phase, as shown in Fig. 6B, was referenced to the phase of the malleus/incus complex. The progressive phase lag with increasing frequency is consistent with a traveling wave propagating along the length of the cochlea [1].

## 4. Discussion

Herein, we demonstrate that spectral domain OCT can image the interior structures of the living mouse cochlea and measure nanoscale vibrations within the organ of Corti near the cochlear apex. The CF of the region we recorded from was ~9 kHz, which is close to the predicted characteristic frequency for a region approximately a half turn from the mouse helicotrema, the apical end of the cochlea [23]. Furthermore, the shape of the magnitude and phase plots and the 0.13 dB/dB slope of the stimulus intensity versus vibration magnitude at the CF are comparable to previous measurements from the cochlear base of larger mammals [6,7]. This suggests that the apical turn of the mouse cochlea functions similarly to the basal turn of larger rodents. As well, this research now opens up the possibility of investigating cochlear mechanics in transgenic mouse models of hearing loss.

An important consideration for this type of experiment is to manage animal motion. It takes less than three seconds to collect and save the raw camera data of a single trial of 10,000 A-lines at a specific stimulus frequency and amplitude. For our *in vivo* experiment, we chose to process and average the data before saving to reduce hard-drive usage. It took approximately an hour to collect the data at six different stimulus levels and nine different frequencies with the requisite averages. When fixed in place and anesthetized, the head of the mouse remained still over this time period, as assessed by viewing B-scans of the cochlea before and after the experiment. Nevertheless, it is certainly possible that small (<10 μm) movements could have occurred during this time. However, averaging the data over multiple depths was done to reduce the impact of such changes.

Ideally, we would like to analyze vibration depth-by-depth across multiple A-lines covering the entire organ of Corti. Two OCT studies in the opened cochlea of larger mammals have shown that this kind of information can provide insights into the interplay between the different components of the organ of Corti [17,18]. Because averaging multiple trials may still be needed to lower the noise threshold of vibration measurements inside the unopened cochlea, managing experimental times will be a concern. The fact that the surgical technique we used does not require opening the cochlea is a benefit however. It reduces the risk of trauma affecting experimental results, and thus, is expected to lead to increased reliability of our experimental preparations.

## 5. Conclusion

Spectral domain OCT can be used to measure vibrations at the apex of unopened mouse cochlea *in vivo*. Since the data are comparable to that measured with LDV without the risks associated with opening the cochlea, OCT represents a new standard for the measurement of vibrations of intracochlear tissues.

## Acknowledgments

We would like to thank Drs. Richard Baraniuk, William Brownell, Stefan Heller, Sunil Puria, Robert Raphael, Anthony Ricci, Peter Saggau, and Tomasz Tkaczyk for helpful advice. The artwork is by Scott Weldon. This project was funded by DoD W81XWH-11-2-0004.