Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Physiology

# Cochlear mechanics: new insights from vibrometry and optical coherence tomography

Elizabeth S Olson[1,2] and C Elliott Strimbu[1]

The cochlea is a complex biological machine that transduces sound-induced mechanical vibrations to neural signals. Hair cells within the sensory tissue of the cochlea transduce vibrations into electrical signals, and exert electromechanical feedback that enhances the passive frequency separation provided by the cochlea's traveling wave mechanics; this enhancement is termed cochlear amplification. The vibration of the sensory tissue has been studied with many techniques, and the current state of the art is optical coherence tomography (OCT). The OCT technique allows for motion of intra-organ structures to be measured *in vivo* at many layers within the sensory tissue, at several angles and in previously under-explored species. OCT-based observations are already impacting our understanding of hair cell excitation and cochlear amplification.

**Addresses**
[1] Department of Otolaryngolgy Head and Neck Surgery, Vagelos College of Physicians and Surgeons, Columbia University, 630 W 168th St, New York, NY 10032, United States
[2] Department Biomedical Engineering, Columbia University, 351 Engineering Terrace, 1210 Amsterdam Avenue, New York, NY 10027, United States

Corresponding author: Olson, Elizabeth S (eao2004@columbia.edu)

**Current Opinion in Physiology** 2020, **18**:xx–yy

This review comes from a themed issue on **Physiology of hearing**

Edited by **Paul Fuchs** and **Barbara Shinn-Cunningham**

https://doi.org/10.1016/j.cophys.2020.08.022



## Early and recent techniques of cochlear vibrometry

The cellular component of the cochlea's sensory tissue, termed the organ of Corti (OoC), is a long narrow strip, bounded by two acellular structures, the basilar and tectorial membranes (BM and TM) and surrounded by fluid chambers. A sound stimulus enters the cochlea at the basal end, and makes its way to frequency-dependent locations by means of a fluid-mechanical traveling wave. The leading actors of hearing are the hair cells (HC), whose stereocilia 'hair' extend from the apical (top) surface of the cell body (at the reticular lamina, RL) to reach or nearly reach the adjacent surface of the TM. Relative motion between the RL and the TM pivots the stereocilia, opening/closing transduction channels, modulating current flow through the channels and thus HC voltage, which leads to transmitter release to the numerous afferent neurons contacting each inner HC. Recent reviews of cochlear and hair cell processing include [1–4]. The sensory tissue of the cochlea vibrates at the frequencies of incoming sound, which extends to 20 kHz for human hearing and to 60 kHz and above for many mammals. Soft to loud sounds range in pressure amplitude from 20 μPa to 2 Pa, corresponding to 0–100 dB SPL with the definition dB SPL = 20 log($P$/20 μPa). The amplitude of the motions for moderate sound pressure levels is on the order of 10 nm. Thus, the challenge of cochlear mechanics is to measure nm-scale motions at frequencies approaching 100 kHz, within a nearly transparent tissue.

Detecting sound-induced cochlear motion was initially done with microscopy coupled to stroboscopic techniques [5], where sampling frequencies can be relatively low (less than the sound frequency). Microscopic/stroboscopic techniques have advanced and have been used in *in vitro* preparations to simultaneously measure multiple motions within the organ of Corti [6], and even stereocilia deflections [7••].

Paradigm-changing advances in vibrometry were made using the Mössbauer technique, in which a radioactive source was placed on the BM and gamma radiation was detected. When the BM moved, the radiation energy was shifted due to a Doppler shift [8,9]. This is a complicated and nonlinear measurement method, which makes one grateful for the subsequent advances in lasers and optics! However, the method was sensitive enough so that a noise floor of ~0.03 mm/s could be attained, corresponding to a displacement of 5 nm at 1000 Hz [9]. Rhode reported active nonlinearity in BM motion using the Mössbauer technique in 1971 [8] and cochlear amplification and its physiological vulnerability were reaffirmed throughout that decade. The term 'cochlear amplification' primarily refers to the healthy, *in vivo* cochlea's active and nonlinear boosting of mechanical responses — by a factor of up to 1000 — at frequencies close to a location's 'characteristic frequency' (CF). In the context of sensory tissue motion, CF refers to the frequency at which the motion reaches its maximum at the measured location, when stimulated with soft tones. CF is also used for neural responses, and signifies the sound frequency that

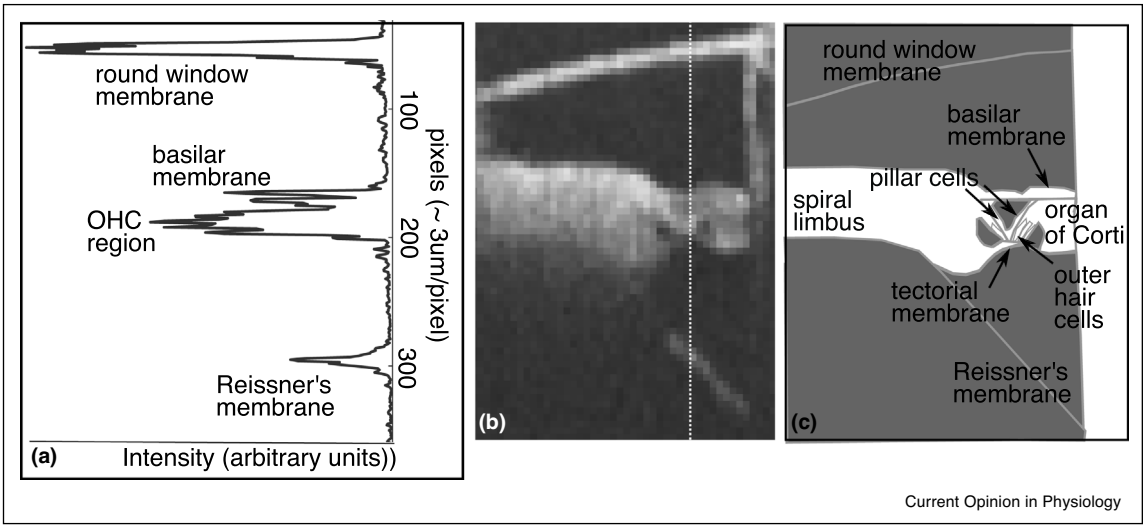
most readily increases a neuron's firing rate. The major findings of cochlear activity, cochlear nonlinearity and the similarity of BM motion tuning and auditory nerve frequency-tuning-curves, were made with the Mössbauer technique [8,9] and corroborated with interferometric techniques [10,11]. Another early cochlear vibration technique was the capacitive probe [12] and a more recent technique was the fiber optic sensor used to measure fluid pressure and motion at the BM [13,14].

Laser interferometry was introduced to cochlear mechanics in the early 1980s [15]. Interferometry uses the constructive and destructive interference of two light beams — reference and incident beams. In homodyne interferometry the read-out is light power [16]. It is a problematically nonlinear measurement method that was soon replaced by heterodyne interferometry, which monitors the phase of the interference signal from the two beams. Heterodyne interferometry became the go-to method of cochlear vibrometry for many years, and the commercial system made by Polytec is a staple of many auditory physiology labs [17,18]. The Polytec vibrometer is a point-and-shoot system, whose read-out is typically directly proportional to velocity. A limitation of standard laser interferometry is that the laser is focused on the sensory tissue surface, which means that in the high frequency base of the cochlea only BM motion was measured, and in the low frequency apex, the motion of the surface of the OoC or TM was measured. This limitation is due to the surfaces that could be accessed atraumatically either through cochleostomies or the natural opening of the round window. The apical measurements showed less nonlinearity and less tuning than the base. The apical/basal differences were difficult to fully grasp since it was not known to what degree the differences were due to the different longitudinal locations, versus due to the different surfaces of measurement. Basal measurements showed strong and interesting nonlinearity and also had easier anatomical access, so were emphasized. Scores of papers were published with heterodyne interferometry, and some of the most interesting findings were related to aspects of cochlear nonlinearity, such as the mechanical basis for suppression [19,20••,21], and the relationship between mechanics, distortion products and distortion product emissions [22,23] see also [24]. To summarize the history: the Mössbauer technique admitted the age of cochlear nonlinearity, and decades of heterodyne interferometry (1980s–2010) squeezed out almost every drop of nonlinearity that could be found at the basal BM. The degree of similarity between BM and auditory nerve responses was surprising, given the complex OoC anatomy that lay between the BM and the stereocilia, but there were also BM motions that were not similar to auditory nerve responses [20••,25]. The advent of OCT-interferometry allowed for the exploration of the under-explored layers within the OoC, in more animals and in all turns of the cochlea.

## OCT vibrometry in the cochlea

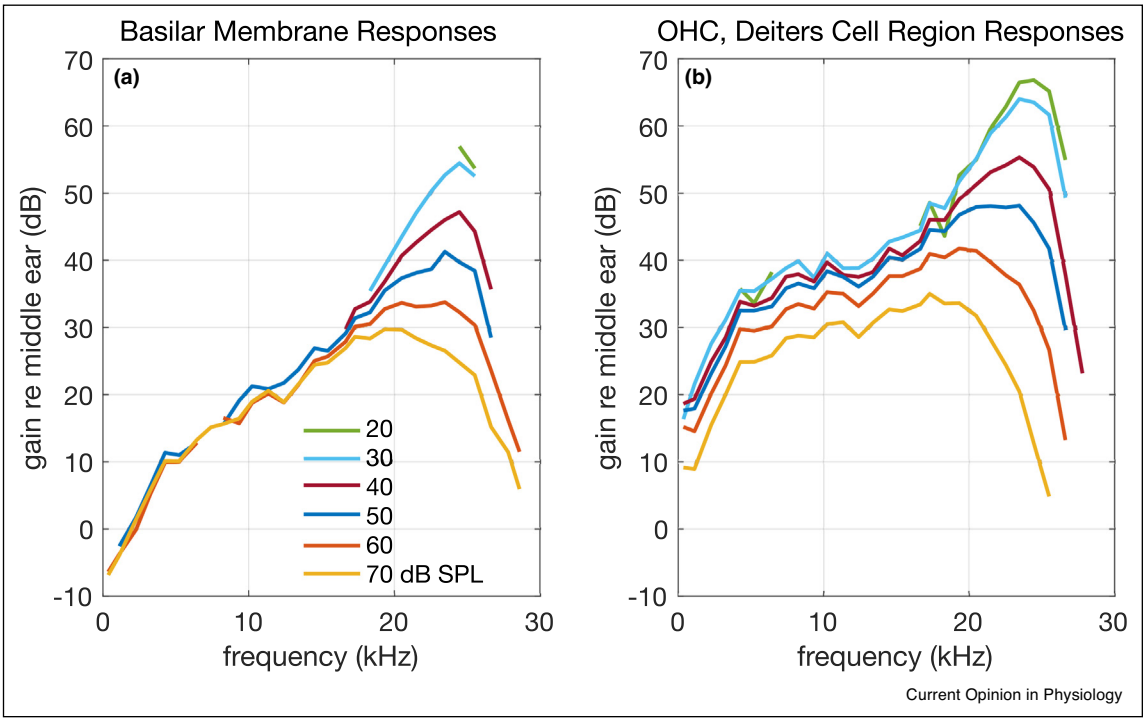
OCT imaging uses a low-coherence infrared light source that can penetrate biological tissue, allowing imaging and interferometric motion measurements at depths of several millimeters. The axial imaging resolution is determined by the bandwidth of the light source (larger bandwidth = better resolution), with resolution values in the micrometer range. Lateral resolution is determined by the lens numerical aperture, as in standard microscopy [26]. In the first applications of OCT to cochlear vibrometry, 'Time-Domain' OCT was used and vibrometry was based on homodyne interferometry [27]. A heterodyne-vibrometry time-domain OCT was designed at about the same time [28]. In time-domain OCT, vibrometry measurements are made at one surface at a time. A significant advance was the introduction of 'Fourier-Domain' OCT (FD-OCT). In the 'Spectral-Domain' version of FD-OCT, SD-OCT [29,30], low-coherence infrared light is mildly focused into the sample so that several millimeters along the axis defined by the beam are within the beam's focus. Sample and reference beams are interfered and this combined beam is then separated by wavelength, and illuminates an array of photodetectors. The pattern of light on these detectors is Fourier transformed into the $x$-domain ($x$ being the axial direction) as $F(x)$. ('Swept-source' OCT is a related FD-OCT modality, in which the light source is swept in time through wavelength and one photodetector is used. The signal is then separated into wavelength components based on timing [31] and Fourier-transformed into the $x$-domain as $F(x)$.) Fourier transforms have terms of magnitude and phase, and the magnitude of $F(x)$ is the 1-D 'image' of the reflectivity of structures along the beam axis (A-scan, as in Figure 1a). (For 2-D imaging, lateral scanning is done with mirrors (B-scan, as in Figure 1b), and a stack of B-scans gives a 3-D image.) To find the motion of structures in the A-scan image, repeated A-scans are taken at a rapid rate ($\sim 10^5$/s). The nm-scale displacements of the sensory tissue are too small to significantly change the A-scan images found with the magnitude of $F(x)$. It is the time variation in the *phase* of the $F(x)$ transform, evaluated at the location corresponding to any given position $x_0$ (selected from the A-scan, for example pixel 190 in Figure 1a), that is used to find the displacement: the axial-direction displacement of the structure at $x_0$ is directly proportional to the time variation in the phase of $F(x_0)$ [32,33]. This phase-based displacement signal is much like the phase-based motion signal of heterodyne interferometry [34], with the same key advantages that it is a linear technique, and can be sensitive down to the sub-nm level. Another powerful advantage is that the motion of all structures along an A-scan are measured simultaneously. Commercially available SD-OCT systems from Thorlabs have brought OCT vibrometry to cochlear physiology labs lacking a full-time optics engineer. However, the Thorlabs system is designed for imaging, and using it for vibrometry requires both

**Figure 1**




**(a)** 'Axial scan' (shortened to 'A-scan') through the organ of Corti of a gerbil. This A-scan was taken along the white dotted line indicated in **(b)**, which shows the corresponding 'Brightness-scan' (shortened to 'B-scan'). **(c)** A cartoon of the B-scan, indicating the organ of Corti structures. This image was taken *in vivo* in the base of a gerbil cochlea, using a Thorlabs Telesto SD-OCT system.

**Figure 2**




Vibrometry data from the base of a gerbil cochlea, taken *in vivo* through the round window membrane, with a Thorlabs Telesto SD-OCT, using custom software developed by M van der Heijden. These results, redrawn from Figure 6 in [37], show that at locations within the OHC, Deiters' cell region of the organ of Corti (termed the 'hotspot' by the authors), nonlinearity extends throughout the frequency range **(b)**. At the BM, the nonlinearity is limited to regions of the peak **(a)**. Data provided by M van der Heijden, and used with authors' permission.

hardware and analytical/software modifications [33,35]. A recent lab-built low-coherence heterodyne interferometer also has the ability to measure vibration at several layers within the cochlea and its results are included below [36].

## Physiological advances

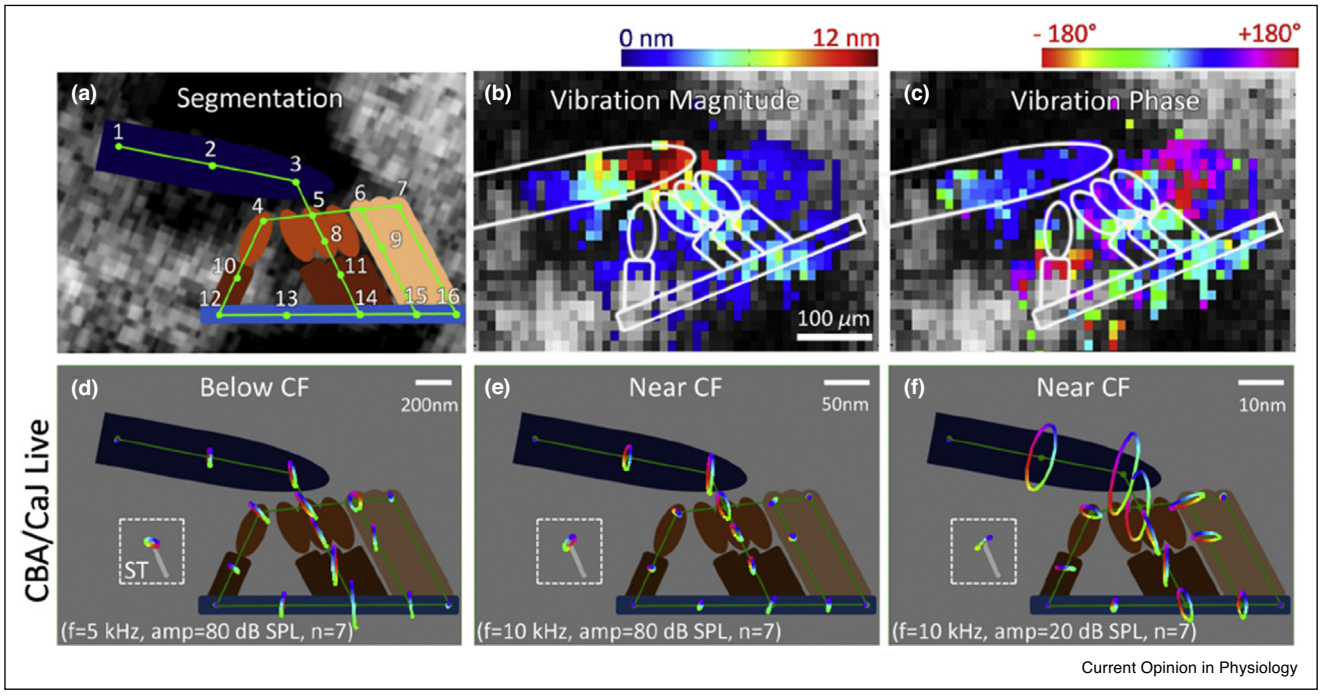
There are two significant benefits of OCT-vibrometry over classic heterodyne vibrometry: the ability to measure different layers within the OoC and the ability to measure through the bone of the cochlear capsule.

The ability to measure within the OoC has exposed significant and unanticipated OoC motions. Motions at the RL and in the OHC/Deiters cell regions exhibit higher amplitudes than at the BM, and enhanced and nonlinear responses in these regions extend to sub-CF frequencies [37–40, 41••] (Figure 2). These findings have stirred up the basic understanding of cochlear amplification, which, in high-frequency regions of the cochlea, had been thought to be limited to the CF peak. The vibrations within the OoC have been analyzed to understand the mechanical processing that gives rise to the frequency/location tuning of cochlear amplification; the results have not yet led to consensus [31,37,42,43••,44]. OCT has allowed for the measurement of traveling wave motion on the TM *in vivo*; measuring vibration with a pure tone stimulus at several longitudinal locations, the TM was observed to possess a larger and sharper peak that reached its maximum further apical than the BM response [31]. The local motions that give rise to stereocilia pivoting and hair cell stimulation have been measured in intact cochleae *in vivo*, by measuring vibrations at one location at two angles and reconstructing the relative motion between the TM and RL [45••] (Figure 3). The sub-CF nonlinearity has been used to probe the intrinsic frequency response of OHC electromechanics [46••].

The ability to measure through cochlear bone has greatly enhanced vibration explorations in genetically modified mice. By combining OCT-vibrometry with histology and microscopy, specific changes in hair cell, hair bundle, or organ of Corti morphology have been mapped to defects or alterations in the mechanical responses [47,48,42]. Pre-OCT studies had already made exciting discoveries in genetically modified mice, but were constrained by the challenging and limited round window approach [49]. The ability to measure through the apical bone has greatly expanded the yield and possibilities for these explorations.

Imaging and doing vibrometry through the cochlear bone has allowed the exploration of apical and basal regions of

**Figure 3**




Vibrometry results from the apex of the mouse cochlea, taken *in vivo* through the bone of the cochlear capsule. These results, from Figure 9 of [45••] were taken with a custom swept-source FD-OCT system developed by the team of J Oghalai and B Applegate. The approach through the bone allowed for motion measurements to be made from two angles, and the 2-dimensional motions within the organ of Corti shown in panels D-F to be determined. The reference includes links to movies that vividly illustrate the motions. Figure used with senior author's permission.

the cochlea in a number of species beyond mice. Traditional interferometry at the apex was restricted to measuring from the apical surface of the OoC, and measurements were made through a cochleostomy that, unless reclosed, disrupted fluid mechanics [50]. In mice, the CF of the apical region is still quite high in frequency (~10 kHz) and the apical responses measured with SD-OCT in mice have been similar to the basal responses of other rodents, with a pronounced CF peak that is strongly nonlinear [42,45••]. In contrast, in gerbil and guinea pig, OCT measurements have observed that the motions in the apical region are broadly tuned or even low-pass [51–53], in agreement with earlier findings from vibration measurements of the OoC surface [50,54]. To date, measurements through bone in these mammals lack well-resolved imaging, making structures within the OoC difficult to discriminate, and more discoveries will come as the technology improves. Beyond mammals, through-the-bone capability has allowed for vibration measurements in the cochlea of the chicken, finding that mechanical tuning was not actively enhanced in that species [55].

## Future

The development and application of more intense and broader bandwidth light sources will improve axial resolution and vibrometry signal:noise [56]. Axial resolution is also improved by employing a shorter wavelength light source [26]. Lateral resolution can be improved by using a higher numerical aperture objective lens [57,28]. However, this comes at the expense of reduced axial working range and distance and will not be practical for some physiological measurements. OCT systems have been coupled to fiber optic probes, allowing for smaller surgical opening and an ability to access remote locations [58], and are moving OCT-imaging and vibrometry into clinical use in the auditory system [59]. OCT-based vibration measurements have recently been performed on awake, behaving mice, finding that motion differences exist in anesthetized versus awake animals [60]. The adaptation of commercially available OCT instruments, such as the Thorlabs series, has brought OCT vibrometry to many cochlear mechanics groups. The ability to measure deep within the OoC allows us to connect the macromechanics of sensory tissue motion to the micromechanics of hair cell stimulation, and further weave the picture of cochlear operation.

## Funding

The authors are supported by an NIH/NIDCD grant to EO and the Emil Capita Foundation.

## Conflict of interest statement

Nothing declared.

## Acknowledgements

We thank Marcel van der Heijden and coauthors for the data shown in Figure 2 of [37] and John Oghalai and coauthors for the use of Figure 3 in [45••].

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- •• of outstanding interest

1. Fettiplace R: **Hair cell transduction, tuning, and synaptic transmission in the mammalian cochlea**. *Comprehens Physiol* 2017, **7**:1197-1227 http://dx.doi.org/10.1002/cphy.c160049.

2. Olson E: *Mechanics of the Cochlea, Reference Module in Neuroscience and Biobehavioral Psychology*. Amsterdam, Netherlands: Elsevier; 2020 http://dx.doi.org/10.1016/B978-0-12-809324-5.24124-2.

3. Ó Maoiléidigh D, Ricci A: **A bundle of mechanisms: inner-ear hair-cell mechanotransduction**. *Trends Neurosci* 2019, **42**:221-236 http://dx.doi.org/10.1016/j.tins.2018.12.006.

4. Ashmore J: **Outer hair cells and electromotility**. *Cold Spring Harbor Perspect Med* 2019, **9** a033522:1–10.

5. von Békésy G, Wever EG: *Experiments in Hearing*. New York: McGraw-Hill; 1960.

6. Karavitaki KD, Mountain DC: **Evidence for outer hair cell driven oscillatory fluid flow in the tunnel of Corti**. *Biophys J* 2007, **92**:3284-3293 http://dx.doi.org/10.1529/biophysj.106.084087.

7. •• Fridberger A, Tomo I, Ulfendahl M, Boutet de Monvel J: **Imaging hair cell transduction at the speed of sound: dynamic behavior of mammalian stereocilia**. *Proc Natl Acad Sci U S A* 2006, **103**:1918-1923 http://dx.doi.org/10.1073/pnas.0507231103
This study used time-resolved confocal imaging to measure both inner and outer hair cell stereocilia deflections *in situ* at the cochlear apex using guinea pig temporal bone preparations. The low ratio of stereocilia deflections to reticular lamina displacements suggested that amplification of the bundle motion may occur *in vivo*.

8. Rhode WS: **Observations of the vibration of the basilar membrane in squirrel monkeys using the Mössbauer technique**. *J Acoust Soc Am* 1971, **49**:1218-1231 http://dx.doi.org/10.1121/1.1912485.

9. Sellick PM, Patuzzi R, Johnstone BM: **Measurement of basilar membrane motion in the guinea pig using the Mössbauer technique**. *J Acoust Soc Am* 1982, **72**:131-141 http://dx.doi.org/10.1121/1.387996.

10. Khanna S, Leonard D: **Basilar membrane tuning in the cat cochlea**. *Science* 1982, **215**:305-306 http://dx.doi.org/10.1126/science.7053580.

11. Narayan SS, Temchin AN, Recio A, Ruggero MA: **Frequency tuning of basilar membrane and auditory nerve fibers in the same cochleae**. *Science* 1998, **282**:1882-1884 http://dx.doi.org/10.1126/science.282.5395.1882.

12. Wilson JP, Johnstone JR: **Basilar membrane and middle ear vibration in guinea pig measured by capacitive probe**. *J Acoust Soc Am* 1975, **57**:705-723 http://dx.doi.org/10.1121/1.380472.

13. Olson ES: **Direct measurement of intra-cochlear pressure waves**. *Nature* 1999, **402**:526-529 http://dx.doi.org/10.1038/990092.

14. Dong W, Olson ES: **Detection of cochlear amplification and its activation**. *Biophys J* 2013, **105**:1067-1078 http://dx.doi.org/10.1016/j.bpj.2013.06.049.

15. Nuttall AL, Fridberger A: **Instrumentation for studies of cochlear mechanics: from von békésy forward**. *Hear Res* 2012, **293**:3-11 http://dx.doi.org/10.1016/j.heares.2012.08.009.

16. Khanna S, Johnson G, Jacobs J: **Homodyne interferometer for basilar membrane vibration measurements. II. Hardware and techniques**. *Hear Res* 1986, **23**:27-36 http://dx.doi.org/10.1016/0378-5955(86)90173-5.

17. Ruggero MA, Rich NC: **Application of a commercially-manufactured Doppler-shift laser velocimeter to the measurement of basilar-membrane vibration**. *Hear Res* 1991, **51**:215-230 http://dx.doi.org/10.1016/0378-5955(91)90038-b.

18. Nuttall A, Dolan D, Avinash G: **Laser doppler velocimetry of basilar membrane vibration**. *Hear Res* 1991, **51**:203-213 http://dx.doi.org/10.1016/0378-5955(91)90037-A.

19. Ruggero MA, Robles L, Rich NC: **Two-tone suppression in the basilar membrane of the cochlea: mechanical basis of auditory-nerve rate suppression**. *J Neurophysiol* 1992, **68**:1087-1099 http://dx.doi.org/10.1152/jn.1992.68.4.1087.

20. •• Geisler CD, Nuttall AL: **Two-tone suppression of basilar membrane vibrations in the base of the guinea pig cochlea using low-side suppressors**. *J Acoust Soc Am* 1997, **102**:430-440 http://dx.doi.org/10.1121/1.419765
This study showed that the known low-side suppression of basal auditory nerve fibers cannot be explained by BM suppression, and hypothesized that a high-pass filter is located somewhere between the BM and the primary auditory neurons. The advent of OCT vibrometry gives hope for identifying a mechanical source of this high-pass filter.

21. Versteegh CPC, Van der Heijden M: **The spatial buildup of compression and suppression in the mammalian cochlea**. *J Assoc Res Otolaryngol* 2013, **14**:523-545 http://dx.doi.org/10.1007/s10162-013-0393-0.

22. Robles L, Ruggero MA, Rich NC: **Two-tone distortion in the basilar membrane of the cochlea**. *Nature* 1991, **349**:413-414 http://dx.doi.org/10.1038/349413a0.

23. He W, Fridberger A, Porsov E, Ren T: **Fast reverse propagation of sound in the living cochlea**. *Biophys J* 2010, **98**:2497-2505 http://dx.doi.org/10.1016/j.bpj.2010.03.003.

24. Dong W, Olson ES: **Supporting evidence for reverse cochlear traveling waves**. *J Acoust Soc Am* 2008, **123**:222-240 http://dx.doi.org/10.1121/1.2816566.

25. Lin T, Guinan JJ: **Time-frequency analysis of auditory-nerve-fiber and basilar-membrane click responses reveal glide irregularities and non-characteristic-frequency skirts**. *J Acoust Soc Am* 2004, **116**:405-416 http://dx.doi.org/10.1121/1.1753294.

26. Izatt J, Choma M: **Theory of optical coherence tomography**. In *Optical Coherence Tomography Technology and Applications*. Edited by Drexler W, Fujimoto JG. Berlin, Heidelberg: Springer; 2008:47-72.

27. Chen F, Choudhury N, Zheng J, Matthews SK, Nuttall AL, Jacques SL: **In vivo imaging and low-coherence interferometry of organ of Corti vibration**. *J Biomed Opt* 2007, **12**:1-9 http://dx.doi.org/10.1117/1.2717134.

28. Hong SS, Freeman DM: **Doppler optical coherence microscopy for studies of cochlear mechanics**. *J Biomed Opt* 2006, **11**:1-5 http://dx.doi.org/10.1117/1.2358702.

29. Ramamoorthy S, Zhang Y, Petrie T, Chen F, Subhash HM, Choudhury N, Wang R, Jacques SL, Nuttall AL: **Measurement of in vivo basal-turn vibrations of the organ of Corti using phase-sensitive Fourier domain optical coherence tomography**. *Photonic Therapeutics and Diagnostics IX, volume 8565*. International Society for Optics and Photonics, SPIE; 2013:279-283 http://dx.doi.org/10.1117/12.2009260.

30. Gao SS, Raphael PD, Wang R, Park J, Xia A, Applegate BE, Oghalai JS: **In vivo vibrometry inside the apex of the mouse cochlea using spectral domain optical coherence tomography**. *Biomed Opt Express* 2013, **4**:230-240 http://dx.doi.org/10.1364/BOE.4.00230.

31. Lee HY, Raphael PD, Park J, Ellerbee AK, Applegate BE, Oghalai JS: **Noninvasive in vivo imaging reveals differences between tectorial membrane and basilar membrane traveling waves in the mouse cochlea**. *Proc Natl Acad Sci U S A* 2015, **112**:3128-3133 http://dx.doi.org/10.1073/pnas.1500038112.

32. Choma MA, Ellerbee AK, Yang C, Creazzo TL, Izatt JA: **Spectral-domain phase microscopy**. *Opt Lett* 2005, **30**:1162-1164 http://dx.doi.org/10.1364/ol.30.001162.

33. Jacques SL, Ramamoorthy S, Petrie TC, Nuttall AL: **A concise algorithm for detection of vibration from Fourier domain OCT**. In *Photonic Therapeutics and Diagnostics IX, vol 8565.* Edited by Mandelis A, Wong BJ., JFIMD, Mahadevan-Jansen A, Jansen ED, HHMD, Madsen SJ, Kang HW, BEKMD, Kollias N, Choi B, Zeng H, Suter MJ, Lam S, Brenner M, KWGMD, GJTMD, Marcu L. International Society for Optics and Photonics, SPIE; 2013:330-335 http://dx.doi.org/10.1117/12.2008966.

34. Lin NC, Hendon CP, Olson ES: **Signal competition in optical coherence tomography and its relevance for cochlear vibrometry**. *J Acoust Soc Am* 2017, **141**:395 http://dx.doi.org/10.1121/1.4973867.

35. Lin NC, Strimbu CE, Hendon CP, Olson ES: **Adapting a commercial spectral domain optical coherence tomography system for time-locked displacement and physiological measurements**. In In *To the Ear and Back Again — Advances in Auditory Biophysics: Proceedings of the 13th Mechanics of Hearing Workshop, vol 1965.* Edited by Bergevin C, Puria S. *To the Ear and Back Again — Advances in Auditory Biophysics: Proceedings of the 13th Mechanics of Hearing Workshop, vol 1965* American Institute of Physics; 2018:080004 http://dx.doi.org/10.1063/1.5038488.

36. Ren T, He W, Barr-Gillespie PG: **Reverse transduction measured in the living cochlea by low-coherence heterodyne interferometry**. *Nat Commun* 2016, **7**:10282 http://dx.doi.org/10.1038/ncomms10282.

37. Cooper NP, Vavakou A, van der Heijden M: **Vibration hotspots reveal longitudinal funneling of sound-evoked motion in the mammalian cochlea**. *Nat Commun* 2018, **9**:3054 http://dx.doi.org/10.1038/s41467-018-05483-z.

38. He W, Kemp D, Ren T: **Timing of the reticular lamina and basilar membrane vibration in living gerbil cochleae**. *eLife* 2018, **7**:e37625 http://dx.doi.org/10.7554/eLife.37625.

39. Ren T, He W, Kemp D: **Reticular lamina and basilar membrane vibrations in living mouse cochleae**. *Proc Natl Acad Sci U S A* 2016, **113**:9910-9915 http://dx.doi.org/10.1073/pnas.1607428113.

40. Chen F, Zha D, Fridberger A, Zheng J, Choudhury N, Jacques SL, Wang RK, Shi X, Nuttall AL: **A differentially amplified motion in the ear for near-threshold sound detection**. *Nat Neurosci* 2011, **14**:770-774 http://dx.doi.org/10.1038/nn.2827.

41. •• Fallah E, Strimbu CE, Olson ES: **Nonlinearity and amplification in cochlear responses to single and multi-tone stimuli**. *Hear Res* 2019, **377**:271-281 http://dx.doi.org/10.1016/j.heares.2019.04.001
This study compared OHC extracellular voltage responses and motion responses at the BM and OHC region. The findings suggested that the sub-CF nonlinearity observed in the OHC region represents OHC electromotility and passive saturation of the OHC mechanoelectric transduction current, and is a non-amplifying nonlinearity.

42. Gao SS, Wang R, Raphael PD, Moayedi Y, Groves AK, Zuo J, Applegate BE, Oghalai JS: **Vibration of the organ of Corti within the cochlear apex in mice**. *J Neurophys* 2014, **112**:1192-1204 http://dx.doi.org/10.1152/jn.00306.2014.

43. •• Dewey JB, Applegate BE, Oghalai JS: **Amplification and suppression of traveling waves along the mouse organ of Corti: evidence for spatial variation in the longitudinal coupling of outer hair cell-generated forces**. *J Neurosci* 2019, **39**:1805 http://dx.doi.org/10.1523/JNEUROSCI.2608-18.2019
This paper reported on two-tone suppression at the RL and on the BM, and compared sub-CF and CF responses. Near-CF responses on the RL and BM showed strongest suppression by tones of frequency higher than CF (in harmony with earlier findings on the BM), but sub-CF responses on the RL showed strongest suppression by tones closest to the suppressed tone frequency. They concluded that a spatial buildup of amplification was only observed in the CF region.

44. He W, Kemp D, Ren T: **Timing of the reticular lamina and basilar membrane vibration in living gerbil cochleae**. *eLife* 2018, **7**:1-17 http://dx.doi.org/10.7554/eLife.37625.

45. •• Lee HY, Raphael PD, Xia A, Kim J, Grillet N, Applegate BE, Ellerbee Bowden AK, Oghalai JS: **Two-dimensional cochlear micromechanics measured in vivo demonstrate radial tuning within the mouse organ of Corti**. *J Neurosci* 2016, **36**:8160-8173 http://dx.doi.org/10.1523/JNEUROSCI.1157-16.2016

This study reported that radial motions of the TM and RL were tuned relative to BM transverse motion. Significant aspects of the 2-D motion were retained in dead preparations, and the authors concluded that the micromechanical motion is substantially governed by passive mechanics.

46. •• Vavakou A, Cooper NP, van der Heijden M: **The frequency limit of outer hair cell motility measured in vivo**. *eLife* 2019, **8**:e47667 http://dx.doi.org/10.7554/eLife.47667
This study used distortion measured in sub-CF vibration responses in the OHC region to quantify the low-pass characteristic of OHC electromotility. They found that the low-pass corner frequency (where responses are reduced by 3 dB) was at ∼3 kHz, where the CF was 13–25 kHz. They concluded that this low-pass characteristic is problematic for theories that include cycle-by-cycle forcing by OHCs.

47. Dewey JB, Xia A, Müller U, Belyantseva IA, Applegate BE, Oghalai JS: **Mammalian auditory hair cell bundle stiffness affects frequency tuning by increasing coupling along the length of the cochlea**. *Cell Rep* 2018, **23**:2915-2927 http://dx.doi.org/10.1016/j.celrep.2018.05.024.

48. Du T-T, Dewey JB, Wagner EL, Cui R, Heo J, Park J-J, Francis SP, Perez-Reyes E, Guillot SJ, Sherman NE, Xu W, Oghalai JS, Kachar B, Shin J-B: **Lmo7 deficiency reveals the significance of the cuticular plate for hearing function**. *Nat Commun* 2019, **10**:1117 http://dx.doi.org/10.1038/s41467-019-09074-4.

49. Russell I, Legan P, Lukashkina V, Lukashkin A, Goodyear R, Richardson G: **Sharpened cochlear tuning in a mouse with a genetically modified tectorial membrane**. *Nat Neurosci* 2007, **10**:215-223.

50. Dong W, Cooper N: **An experimental study into the acousto-mechanical effects of invading the cochlea**. *J R Soc Interface* 2006, **3**:561-571 http://dx.doi.org/10.1098/rsif.2006.0117.

51. Dong W, Xia A, Raphael PD, Puria S, Applegate B, Oghalai JS: **Organ of corti vibration within the intact gerbil cochlea measured by volumetric optical coherence tomography and vibrometry**. *J Neurophysiol* 2018, **120**:2847-2857 http://dx.doi.org/10.1152/jn.00702.2017.

52. Recio-Spinoso A, Oghalai JS: **Unusual mechanical processing of sounds at the apex of the guinea pig cochlea**. *Hear Res* 2018, **370**:84-93 http://dx.doi.org/10.1016/j.heares.2018.09.009.

53. Warren RL, Ramamoorthy S, Ciganović N, Zhang Y, Wilson TM, Petrie T, Wang RK, Jacques SL, Reichenbach T, Nuttall AL, Fridberger A: **Minimal basilar membrane motion in low-frequency hearing**. *Proc Natl Acad Sci U S A* 2016, **113**:E4304-E4310 http://dx.doi.org/10.1073/pnas.1606317113.

54. Khanna S, Hao L: **Amplification in the apical turn of the cochlea with negative feedback**. *Hear Res* 2000, **149**:55-76 http://dx.doi.org/10.1038/nn1828.

55. Xia A, Liu X, Raphael PD, Applegate BE, Oghalai JS: **Hair cell force generation does not amplify or tune vibrations within the chicken basilar papilla**. *Nat Commun* 2016, **7**:13133 http://dx.doi.org/10.1038/ncomms13133.

56. An L, Qin J, Zhi Z, Wang R: **Ultra high resolution ultra high sensitive optical micro-angiography based on super continuum light source**. In *Optical Coherence Tomography and Coherence Domain Optical Methods in Biomedicine XV, vol 7889.* Edited by Fujimoto JG, Izatt JA, Tuchin VV. International Society for Optics and Photonics, SPIE; 2011:180-184 http://dx.doi.org/10.1117/12.875365.

57. Jabeen T, Holt JC, Becker JR, Nam J: **Interactions between passive and active vibrations in the organ of Corti in vitro**. *Biophys J* 2020, **119**:1-12 http://dx.doi.org/10.1101/2020.01.03.893933.

58. Lin NC, Fallah E, Strimbu CE, Hendon CP, Olson ES: **Scanning optical coherence tomography probe for in vivo imaging and displacement measurements in the cochlea**. *Biomed Opt Express* 2019, **10**:1032-1043 http://dx.doi.org/10.1364/BOE.10.001032.

59. Kim W, Kim S, Oghalai J, Applegate B: **Endoscopic Optical Coherence Tomography enables morphological and subnanometer vibratory imaging of the porcine cochlea through the round window**. *Opt Lett* 2018, **43**:1966-1969 http://dx.doi.org/10.1364/OL.43.001966.

60. Lui CG, Dewey JB, Badash I, Macías-Escrivá FD, Kim W, McGinley MJ, Applegate BE, Oghalai JS: **Organ of corti vibrations in awake mice**. *Association for Research in Otolaryngology, 43rd Annual Midwinter Meeting* 2020:70.