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Optical Coherence Tomography to Measure Sound-Induced Motions Within the Mouse Organ of Corti In Vivo

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Abstract

The measurement of mechanical vibrations within the living cochlea is critical to understanding the first nonlinear steps in auditory processing, hair cell stimulation, and cochlear amplification. However, it has proven to be a challenging endeavor. This chapter describes how optical coherence tomography (OCT) can be used to measure vibrations within the tissues of the organ of Corti. These experimental measurements can be performed within the unopened cochlea of living mice routinely and reliably.

Keywords

Cochlear mechanics; Basilar membrane; Tectorial membrane; Vibrometry; Sound encoding; Biomechanics; In vivo imaging

1 Introduction

Different techniques have been used historically to measure basilar membrane motion.

The measurement of basilar membrane motion was pioneered by von Békésy in the 1950s using stroboscopic illumination [1]. In the post-mortem cochlea of multiple species, he identified the presence of traveling waves. The first vibratory measurements in living animals came in 1967 from Johnstone and Boyle, who studied the guinea pig using the Mössbauer technique [2]. In 1971, Rhode also used this approach, in which a radioactive source was placed on the undersurface of the basilar membrane. He identified that sound-induced vibrations of the squirrel monkey basilar membrane were nonlinear [3]. This nonlinear gain increases the dynamic range of mammalian hearing and is termed cochlear amplification [4].

Laser Doppler vibrometry is primarily for surface measurements. From the 1980s to the late 2000s, laser Doppler vibrometry was the predominant technique for measuring sound-induced vibrations of the basilar membrane. Most commonly, the cochlear base was studied near or at the round window, and the most common animals used were those with large cochleae that protrude prominently into the middle ear space, including the guinea pig, gerbil, and chinchilla. With this technique, the otic capsule bone was opened, a small reflective glass bead was dropped onto the undersurface of the basilar membrane, and the

laser was focused on the bead. Motion-induced Doppler shifts in the reflected light could then be measured.

This approach provided the highest available sensitivity and was used to define most of what is currently known about cochlear amplification (for review, *see* [5]). While it has been possible to collect data using laser Doppler vibrometry without placing a reflective bead [6, 7], this approach is challenging because of the low reflectance of the basilar membrane. In addition, some data has been collected from the cochlear apex [8–10]. However, this method, too, has proven difficult because opening the Reissner's membrane to drop a reflective bead onto the surface of the tectorial membrane can disrupt the endocochlear potential and thus alter cochlear function.

Besides the technical difficulties associated with opening the cochlea, laser Doppler vibrometry has a limitation in that its depth resolution is defined by the numerical aperture (NA) of the objective used to focus the light. However, a long working distance objective, which usually has a relatively low NA, makes it easier to target the laser and thus perform the *in vivo* experiments. This means that without the use of a bead to define the spot being measured, reflections from multiple surfaces along the path of the laser may occur, producing artifacts. With the use of the bead, however, the measurement technique is limited to surface measurements. For this reason and the technical issues explained above, laser Doppler vibrometry has primarily been used to measure basilar membrane motion. The remaining structures within the organ of Corti have essentially been unstudied because of their inaccessibility.

Optical coherence tomography (OCT) is an optical imaging modality that permits depth-resolved measurements and is widely used for imaging the eye and coronary arteries. Recent investigations using animal models of the middle and inner ear illustrate the growing demand for the high fidelity tomographic and vibratory information accessible with OCT for interrogating the function of the ear [11–15]. In 2006, OCT was first used to measure vibrations within the living guinea pig cochlea [16]. Further studies performed within the guinea pig cochlear base after opening the otic capsule bone have been used to identify differences between the vibration of the reticular lamina and of the basilar membrane [17].

OCT fundamentally measures the magnitude and phase of light field reflected from a sample as a function of depth. The magnitude is used to render tomographic images where small changes in refractive index provide contrast. The imaging depth of OCT is both tissue and wavelength dependent, but typically falls in the range of 1–2 mm. The axial and lateral resolutions are typically in the range of 1–15 μm and 10–30 μm , respectively. The phase of the light field may be used to measure small relative changes in refractive index or position of the reflectors in the sample. For applications in the ear, the refractive index is typically assumed to be constant with respect to time; hence, any modulation of the phase is attributed to motion. By monitoring the phase as a function of time, relative motion as small as a few picometers can be measured enabling spatially resolved vibrometry [18]. Note that while the relative motion is resolved in 3D space, the position of the moving tissues is only known to the spatial resolution noted above for the tomographic images and only motion parallel to the illumination beam is measured. In other words, while the relative motion can be known

to a few picometers, the tissue structures that are moving are only typically known to a few microns and only the projection of the motion on the optical axis (parallel to the light illumination) is resolved.

There are several methods for realizing an OCT system based on either time-domain or spectral-domain interferometry. Time-domain methods use a moving reference arm reflector in an interferometer to encode the depth of the tissue reflectors in time. Small relative motion is typically measured by monitoring the Doppler shift of the light returning from the sample. This is analogous to laser Doppler vibrometry except that the position of the moving tissue is spatially resolved. In spectral-domain methods, the depth of the tissue reflectors is encoded in the spatial frequency (wavenumber, k) of the reflected light. A Fourier transform converts from spatial frequency to space. The magnitude of the result is the tomographic image. The phase of the Fourier transform can be monitored as a function of time for vibrometry. The earliest systems developed for OCT typically used time-domain methods, however it has been shown that the spectral-domain methods have an inherent sensitivity advantage of 20–30 dB [19–21].

Spectral interferometry for OCT may be accomplished in one of two ways: a broad wavelength band light source and spectrometer to resolve the light as a function of spatial frequency or a narrow wavelength band light source where the center frequency is swept as a function of time and detected with a photodiode. The former is typically called Fourier-domain OCT (FD-OCT) and the latter is called swept-source OCT (SSOCT).

OCT permits the noninvasive measurement of sound-induced motion within the intact mouse cochlea. A key goal in understanding cochlear mechanics is to measure the motions of the various microstructures that compose the organ of Corti, not just the basilar membrane. These structures are highly organized and presumably work together to develop cochlear amplification. Ideally, these measurements would be made in an intact (i.e., unopened) cochlea to minimize trauma to the cochlea and to permit increased experimental reliability. In addition, the ability to study the mouse cochlea would offer some benefits because transgenic strains with altered biomechanics are available. To achieve these goals, we developed a FD-OCT system that permitted vibrometry within the apical turn of the mouse cochlea [11, 13, 22] and discovered complex micromechanical vibratory characteristics within the supporting cells of the organ of Corti [12]. However, this OCT system was not as sensitive as laser Doppler vibrometry, and therefore averaging of repeated measurements was required in order to achieve a reasonable noise floor. This meant that experimental time was longer than ideal, which is an issue when performing in vivo experiments.

We then developed a new technique based upon SSOCT, termed volumetric optical coherence tomography vibrometry (VOCTV), which overcomes these limitations. The technique offers depth-resolved displacement measurements with sensitivity and speed comparable to laser Doppler vibrometry. Using VOCTV in the mouse cochlea, we measured the traveling wave within both the basilar membrane and the tectorial membrane, and we found key differences that explain unusual features found in auditory nerve single unit recordings [18]. With this approach, we open the middle ear bulla but do not open the cochlea. The success rate for an experiment using VOCTV in our lab is roughly 90 %, and

we routinely perform experiments on 2–3 mice per day. Unsuccessful experiments occur if the mouse dies unexpectedly or if the experimenter inadvertently damages the tympanic membrane or ossicular chain during the surgical exposure.

This chapter describes the dissection process, the imaging process, and the vibratory measurement calculations that we use to make these measurements in living mice. Our current optical setup design has been published [18], and frankly, given the rapid advances in optics, it is already out of date. However, any OCT setup can be used with the animal preparation we describe to make similar recordings.

2 Materials

2.1 Supplies for Mouse Anesthesia

1. We use a combination of ketamine and xylazine to anesthetize the mice. The anesthetic dose for mice is ketamine 100 mg/kg and xylazine 10 mg/kg. We dilute and then mix these two drugs together in the correct proportions so that only one injection needs to be given.
2. 0.9 % NaCl, 100 mg/mL ketamine, 20 mg/mL xylazine.
3. Ketamine working solution: Mix 0.6 mL ketamine with 2.4 mL NaCl to make 3 mL of diluted ketamine (20 mg/mL).
4. Xylazine working solution: Mix 0.05 mL xylazine with 2.94 mL NaCl to make 3 mL of diluted xylazine (2 mg/mL).
5. Final anesthetic solution: Mix diluted ketamine with diluted xylazine to make 6 mL of anesthetic solution at a concentration of 10 mg/mL ketamine and 1 mg/mL xylazine. Store at room temperature.

2.2 Surgical Equipment

2.2.1 Surgical Apparatus

1. Stage with manual 2D movement controls fixed to a vibration isolation table, which is used to adjust the mouse position relative to the dissecting microscope.
2. Thermoregulated heating pad placed on top of stage.
3. Head holder attached to stage (*see Note*¹).
4. 70 % Ethanol to sterilize head holder.
5. Dissecting microscope with attached OCT system positioned over the stage (Fig. 1).
6. Speaker to generate the sound stimuli positioned near the stage.

¹The head holder is necessary to rigidly fix the head in order to reduce movements from other sources that might affect the vibratory measurements.

2.2.2 Surgical Instruments (Fig. 2)

1. Small gauze pads.
2. Cotton balls.
3. Battery-operated cautery tool.
4. Dissection instruments: forceps, scissors, straight pick or needle, scalpel, rongeurs.
5. Methyl methacrylate glue, both the powder and liquid components (Co-Oral-Itte Dental Mfg., Diamond Springs, CA).
6. Small cup or weighing boat and Q-tips to mix glue solution.
7. Anesthetic Monitoring Worksheet.

2.2.3 Optical Equipment

1. Broad band laser, either swept source or wide-bandwidth (e.g., MEMS-VCSEL, Thorlabs, Newton, NJ).
2. Photodetector (e.g., WL-BPD600MA, Wieserlabs).
3. Digitizer (e.g., NI-5772, National Instruments, Austin, TX).
4. Field programmable gate array (FPGA) (e.g., NI-7966R, National Instruments).
5. 2D galvo mirror (e.g., OIM101, Optics In Motion, Long Beach, CA).

3 Methods

3.1 Anesthetization

The institutional animal care and use committee should approve all animal procedures according to the appropriate guidelines.

1. Use adult mice, aged P28 to P42 to perform an intraperitoneal (IP) injection of the proper dose.
2. Weigh mouse and inject proper volume of anesthetic solution based on using 0.1 mL/10 g at the beginning of the experiment.
3. Perform a paw pinch test, to determine whether the mouse needs additional anesthesia by using forceps to gently squeeze the foot. If the leg withdraws, additional anesthesia is required. Do not start surgical procedures until there is no withdrawal.
4. Supplement anesthetic every 30 min, although the time-length may vary depending on each individual animal. Typically we give 0.05 mL of the anesthetic solution.
5. Use an Anesthetic Monitoring Worksheet (Fig. 3) throughout each experiment to document monitoring procedures and all anesthesia injections. Keep these records in a binder for semiannual IACUC inspections.

3.2 Mouse Placement in Head Holder

1. Once fully anesthetized, use scissors to gently remove the scalp between both ears. The left ear will be used as an example. Leave the pinna in place or transect the ear canal and remove the pinna depending on experimental needs.
2. Dry the scalp with a gauze pad, and insert the head into the head holder (Fig. 4a) (*see Note*²).
3. Glue skull to head holder using methyl methacrylate glue (Fig. 4b) and allow it to harden for 5–10 min.
4. Gently rotate the mouse and head holder to expose the left ear.

3.3 Surgical Exposure of Cochlea [23]

1. Remove skin covering the ventral side of the left ear and the left masseter muscle with scissors (Fig. 4c).
2. Remove the submandibular gland and perform a deeper dissection with the scissors and scalpel to divide the edge of the trapezius muscle and the posterior belly of the digastric muscle. Use the cautery where needed to control bleeding. The tympanic bulla then becomes visible (Fig. 4d) (*see Note*²).
3. Remove part of the jaw, in order to view the cochlear apex, by first removing the masseter muscle, which lies on the lateral surface of the mandible, with the cautery. Use the rongeurs to remove the ramus of the mandible (*see Note*³).
4. Open the bulla medial to the tympanic annulus with care (Fig. 4e) by first scraping the bulla with the tip of a small scalpel to soften it. Then, use a straight pick or needle to remove the bone fragments. Do not open the bulla until all bleeding is stopped, since any blood seeping into the middle ear space reduces the quality of the OCT image.
5. Note the cochlea on the floor of the middle ear cavity. Adjust the head holder to tilt the head at roughly a 45° angle, which brings the cochlear apex in line with the optical axis of the microscope and permits imaging of most of the apical turn. The dissection is now complete.

3.4 Imaging: Investigating the Mouse Cochlea

3.4.1 OCT imaging Terminology

1. A-line: Park the *x* and *y* scan mirrors at one position, and collect a line image along the optical path of the laser (*see Note*⁴).

²Take care to prevent damage to structures. The heat of the cautery can potentially damage the bulla and cochlea. In addition, it is critically important to note the white line through the bone of the tympanic bulla, the tympanic annulus, which marks the edge of the tympanic membrane. Care must be taken so that this structure is not damaged when opening the bulla.

³Bleeding can occur from the pterygoid muscles just deep to the mandible. Staying close to the bone of the mandible is the best way to prevent bleeding. Cautery is often ineffective if bleeding starts in this area. Instead, cotton wispes can be used to pack off this region. The venous bleeding will stop with time.

⁴The A-line measures the image intensity versus depth at one *x*, *y* position.

2. B-scan. Move the scan mirrors so that the laser is moved along a linear path, and collect multiple sequential A-lines. Plot these to give a planar image along the x - z axes.
3. C-scan or Volume scan. The scan mirrors are moved to cover the entire x - y range, while collecting A-lines. This scan creates a 3D x - y - z image.
4. M-scan. Park the scan mirrors at one location and collect multiple A-lines over time. The magnitude of the signal from any given voxel does not change, but its phase varies if the structure is vibrating. The vibration of one or more pixels along the z -direction is measured with this technique.
5. BM-scan. Scan mirrors move the laser step-by-step along a linear path, stopping at evenly spaced intervals to collect an M-scan, allowing the measurement of vibrations over a 2D region of space.
6. CM-scan. This scan is similar to a BM-scan, but measures vibration throughout a volume of tissue.

3.4.2 Identifying Regions of Interest

1. Image the cochlea, in order to identify a region of interest for measuring vibrations, by rapidly collecting B-scans with the VOCTV system.
2. Collect B-scans continuously and align the mouse under the system until identifying a mid-modiolar cross-section of the cochlea (*see Note* ⁵).
3. Ensure that the quality of the image is sharp (Fig. 5). We use dispersion compensation to account for differences in the amount of glass in the sample and reference arms of the VOCTV system. We have many different algorithms stored, and the user can switch between them to see which one gives the best image. In addition, adjusting the polarization paddles contained within the optical path of the sample arm may help.
4. Once a region of interest is found, save the image. This image is used later, to show where the measurement was made when analyzing the vibratory data.

3.4.3 Measuring Vibratory Responses from a Single Point

1. Select a measurement location for study on the B-scan image. Commonly, this location is the mid-portion of the basilar membrane; however, one can use any pixel within the image.
2. Present sound stimuli of varying frequencies and intensity to the ear, while collecting M-scans to measure vibratory responses at this point (*see Note* ⁶).
3. Begin collecting data (Fig. 6a), which is completed in ~2–3 min.

⁵To achieve this cross-section, the head holder is physically adjusted and/or the direction of the scan is changed. On our system, a scan rotation angle of -30° works well.

⁶These stimulus parameters need to be set in software. Our standard parameters for measuring vibrations within the apex of the mouse cochlea are to sweep the frequency from 2 to 14 kHz in steps of 0.5 kHz and to sweep the intensity from 10 to 80 dB SPL in steps of 10 dB.

4. Analyze the vibratory responses at the selected point. If desired, vibrations can be processed and analyzed at all points along the A-line as the information is collected simultaneously.

3.4.4 BM-Scans—This process is similar to single-point measurements; however, vibratory data are collected from multiple points.

1. Collect a B-scan image first, by selecting an area for study using the computer mouse. We usually select the organ of Corti, from the osseous spiral lamina to the spiral ligament.
2. Set the step size, which defines the number of points at which the scan mirrors will stop along the scan path, thereby setting the sampling resolution along the horizontal axis. Note that the resolution along the vertical axis (the *z* direction) is determined by the bandwidth of the laser used for the VOCTV system.
3. Set the sound stimulus parameters, which are sequentially repeated for each step in the scanning process. This can dramatically increase the data collection time and it is recommended that the user carefully consider the number of stimulus frequencies and intensities to be studied.
4. Collect the data (Fig. 6b).

3.4.5 CM-Scan—The CM-scan is performed similarly to the BM-scan; however, selecting the region of interest is more complex, because it must be done in 3D. Therefore, this scan is similar to collecting an image Z-stack on a confocal microscope. However, the starting and stopping points of the scan are defined in the *y*-axis rather than the *z*-axis.

1. Collect a volume scan of the cochlea, and then scroll through the B-scans to define the starting and stopping points in the *y*-dimension. The region of interest in the *x* and *z* dimensions is selected with the computer mouse as described above for the BM-scan.
2. Set step sizes for both the *x* and *y* dimensions. We usually use 20 μm .
3. Set sound stimuli parameters.
4. Collect the data.

3.5 Completion of the Experiment

When analyzing the data, it is important to calculate the ratio of the vibratory responses to the intensity of the sound that entered the cochlea. While the frequency response of the speakers are calibrated prior to each experiment, the ear canal, tympanic membrane, and ossicular chain all filter the sound stimuli.

1. Measure the vibratory response of the ossicular chain after all cochlear measurements have been completed. In the mouse, we typically target the laser onto the orbicular apophysis of the malleus.
2. Present all sound frequencies at 80 dB SPL and collect vibratory data. Because the vibration of the ossicular chain is linear, these data can be scaled as needed.

3. Euthanize the mouse at the end of the experiment with an overdose of anesthesia solution and confirm according to guidelines.

3.6 Analysis

3.6.1 Phase Measurements to Displacement to Displacement Magnitude and Phase

The data collected when performing vibrometry with VOCTV is phase versus time. For a single point measurement, this is simply one array. For a BM-scan or CM-scan, there will be an array for each voxel contained within the selected region of interest (*see Note 7*)

1. Perform a Fourier transform to identify the vibratory magnitude and phase at the stimulus frequency (*see Note 8*).
2. Commonly measured characteristics that can be calculated during data analysis include:
 - The Characteristic Frequency (CF) is the frequency of maximal vibration to stimuli of very low intensity (typical 10–20 dB SPL), which defines the measurement location along the cochlear tonotopic map.
 - The Best Frequency (BF) is the maximal frequency of vibration response to the stimulus intensity. At low stimulus intensities, the BF equals the CF; however, the BF shifts to a lower frequency with higher intensity stimuli.
 - The $Q_{10\text{dB}}$ is a quantification of sharpness of tuning. Originally, it was defined for use with auditory nerve tuning curves. However, it can be adapted for use with vibratory measurements by dividing the CF by the bandwidth measured 10 dB below the vibratory magnitude at the CF.
 - The *gain* is calculated by dividing the peak vibratory magnitude at the CF measured in the live animal by peak vibratory magnitude in the dead animal.

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⁷To convert this to displacement, use the following equation:

$$D = \varphi \times \frac{\lambda}{4\pi n} \quad (1)$$

where D is the displacement at each point in time, φ is the phase at each point in time, λ is the center wavelength of the light used, and n is the refractive index of the sample. At 1300 nm, the refractive index of water is ~1.32.

⁸Because of the fixed time delay for sound to propagate from the speaker into the ear, and the filtering response of the middle ear, the phase of intra-cochlear structures are referenced to the phase of the ossicular chain. This is accomplished by subtracting the phase of the ossicular chain from the phase of the cochlear structure used in measuring.

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Fig. 1.
Our experimental setup containing the VOCTV system

Fig. 2.

Surgical instruments: battery-operated cautery tool, forceps, glue, head holder, needle, one cotton ball, one small gauze pad, rongeur, scalpel, and scissors

Mouse Anesthetic Monitoring Form

Date:

Researcher:

PI:

APLAC #:

Induction Anesthesia:

Ketamine: 100 mg/kg

Xylazine: 10 mg/kg

Time	Anesthesia Given (Induction)	Level of Anesthesia (Assessed by signs of movement or withdrawal reflex)	Respiratory Rate & Pulse (Assessed visually)	Body Temperature (Assessed with heating blanket temperature probe)	Color (View skin around eyes and paws)
0:00	_____ ml (_____ g)	☐ OK ☐ Sacrifice ☐ Not OK ☐ Recover	☐ OK ☐ Not OK	☐ OK ☐ Not OK	☐ OK ☐ Not OK
0:15	_____ ml	☐ OK ☐ Sacrifice ☐ Not OK ☐ Recover	☐ OK ☐ Not OK	☐ OK ☐ Not OK	☐ OK ☐ Not OK
0:30	_____ ml	☐ OK ☐ Sacrifice ☐ Not OK ☐ Recover	☐ OK ☐ Not OK	☐ OK ☐ Not OK	☐ OK ☐ Not OK
0:45	_____ ml	☐ OK ☐ Sacrifice ☐ Not OK ☐ Recover	☐ OK ☐ Not OK	☐ OK ☐ Not OK	☐ OK ☐ Not OK
1:00	_____ ml	☐ OK ☐ Sacrifice ☐ Not OK ☐ Recover	☐ OK ☐ Not OK	☐ OK ☐ Not OK	☐ OK ☐ Not OK
1:15	_____ ml	☐ OK ☐ Sacrifice ☐ Not OK ☐ Recover	☐ OK ☐ Not OK	☐ OK ☐ Not OK	☐ OK ☐ Not OK
1:30	_____ ml	☐ OK ☐ Sacrifice ☐ Not OK ☐ Recover	☐ OK ☐ Not OK	☐ OK ☐ Not OK	☐ OK ☐ Not OK
1:45	_____ ml	☐ OK ☐ Sacrifice ☐ Not OK ☐ Recover	☐ OK ☐ Not OK	☐ OK ☐ Not OK	☐ OK ☐ Not OK
2:00	_____ ml	☐ OK ☐ Sacrifice ☐ Not OK ☐ Recover	☐ OK ☐ Not OK	☐ OK ☐ Not OK	☐ OK ☐ Not OK
2:15	_____ ml	☐ OK ☐ Sacrifice ☐ Not OK ☐ Recover	☐ OK ☐ Not OK	☐ OK ☐ Not OK	☐ OK ☐ Not OK
2:30	_____ ml	☐ OK ☐ Sacrifice ☐ Not OK ☐ Recover	☐ OK ☐ Not OK	☐ OK ☐ Not OK	☐ OK ☐ Not OK
2:45	_____ ml	☐ OK ☐ Sacrifice ☐ Not OK ☐ Recover	☐ OK ☐ Not OK	☐ OK ☐ Not OK	☐ OK ☐ Not OK
3:00	_____ ml	☐ OK ☐ Sacrifice ☐ Not OK ☐ Recover	☐ OK ☐ Not OK	☐ OK ☐ Not OK	☐ OK ☐ Not OK
3:15	_____ ml	☐ OK ☐ Sacrifice ☐ Not OK ☐ Recover	☐ OK ☐ Not OK	☐ OK ☐ Not OK	☐ OK ☐ Not OK
3:30	_____ ml	☐ OK ☐ Sacrifice ☐ Not OK ☐ Recover	☐ OK ☐ Not OK	☐ OK ☐ Not OK	☐ OK ☐ Not OK

Fig. 3.

Mouse anesthetic monitoring form

Fig. 4.

Surgical exposure of the mouse cochlear apex. (a) The anesthetized mouse is placed in the head holder. (b) The head is glued to the head holder to provide rigid fixation. (c) The ventral approach to the tympanic bulla. The masseter muscle is noted (*arrow*). (d) The tympanic bulla has been exposed. The tympanic annulus (*arrow*) defines the attachment of the tympanic membrane. (e) After opening the bulla, the cochlea (*arrow*) is barely visible on the floor of the middle ear cavity

Fig. 5.

B-scans of the cochlear apex. **(a)** This image demonstrates poor dispersion compensation. Reflections at the boundary between the air and the otic capsule bone are particularly noticeable (*arrow*). **(b)** A B-scan after application of appropriate dispersion compensation. Scala Vestibuli (*SV*), Scala Media (*SM*), Scala Tympani (*ST*), Tectorial Membrane (*TM*), Basilar Membrane (*BM*). Scale bar 100 μm

Fig. 6. Vibratory responses. (a) A single point tuning curve from the mouse basilar membrane. (b) ABM scan from the mouse organ of Corti. Only magnitude data are shown in these representative examples. Reproduced from Ref. [18] with permission