

# Basilar Membrane Properties And Measurement For Tonotopic Frequency Map

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**Abstract:** The cochlear frequency-place map is believed to be an important determinant of the frequencies that a species can hear as well as the bandwidth of cochlear filters. Both features impact an animal's ability to detect biologically significant sounds in noise. The cochlear frequency-place map is created partially a stiffness gradient in the basilar membrane (BM) in which stiff regions respond best to high frequencies and more compliant regions respond best to low frequencies. BM mass doesn't play significant role infrequency –place map as it remains constant. Due to the position of BM in cochlea, the BM stiffness measurements are difficult. Both hemi cochlea and in-vivo preparations are carried out worldwide for BM stiffness measurements along longitudinal length and radial length. Both methods can be specific for different species and utmost care is exercised to maintain the integrity of the tissues under consideration. BM mechanical properties can change during its early developmental stage gets matured in adult and degrade again due to aging, exhibiting change infrequency –place map. Also BM exhibits longitudinal coupling which make complex energy transfers in cochlear duct during wave propagation. BM mechanical properties, its measurement techniques are presented in this paper. Alteration in BM mechanics by any factor may lead to hearing impairments

## I. INTRODUCTION

Basilar membrane (BM) lies within the cochlea and its mechanical properties are dominating factors in generating the tonotopic frequency map. Worldwide attempts are made to study the mechanical properties of BM .in this paper we introduce various mechanical properties of BM and its measurement techniques. Our paper is arranged in following manner.

**Section I:** This section describes various attributes of BM, such as its position inside cochlea and its typical dimensions. It explains various zones of BM and presence of radial tension. It explains the function of BM, presence of hair cells, its mathematical model and tonotopic response.

**Section II:** This section explains the two techniques for BM stiffness measurement. Both hemicochlea and in-vivo techniques require special care to maintain the integrity of the tissues for practical readings. It also explains the difference between hemicochlea and in-vivo methods.

**Section III:** This section explains the role of BM stiffness in generation of frequency place map. It capture the overall dynamics of cochlea in an equation explaining

### Section I: BM PROPERTIES

#### Cochlear structure

The cochlea consists of a coiled labyrinth, like a snail, which is about 10mm across and has about 2.5 turns in humans, embedded in the temporal base of the skull. It is filled with fluid and divided into three main fluid chambers, as described below and as shown in the Figure 2It shows that the scala vestibuli is at the top, which is separated from the scala media

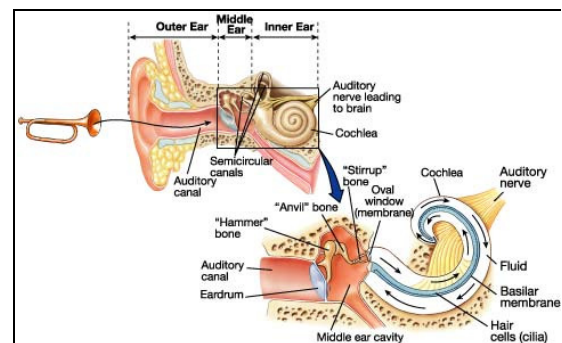
by a thin flexible partition called Reissner's membrane, and the scala media is separated from the scale tympani at the bottom by a rigid partition that includes a more flexible section, called the BM.

#### BM position within cochlea

As shown in Figure1 the BM within the [cochlea](#) of the [inner ear](#) is a stiff structural element that separates two liquid-filled tubes that run along the coil of the cochlea, the [scala media](#) and the [scala tympani](#). The basilar membrane is a pseudo-resonant structure that varies in width and stiffness. The BM has different properties (width, stiffness, mass, damping, and the dimensions of the ducts that it couples to) at different points along its length.

#### Typical BM dimensions

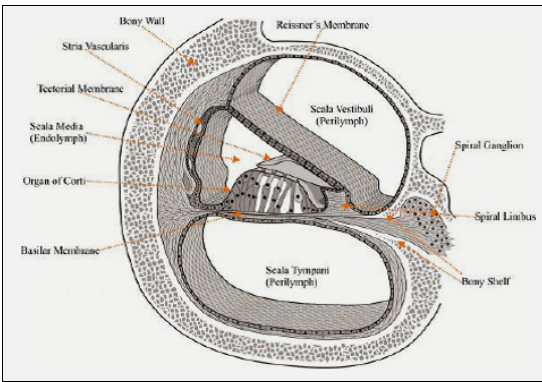
The motion of the basilar membrane is generally described as a traveling wave. The parameters of the membrane at a given point along its length determine its characteristic frequency (CF), the frequency at which it is most sensitive to sound vibrations. The basilar membrane is widest (0.42–0.65 mm) and least stiff at the apex of the cochlea, and narrowest (0.08–0.16 mm) and most stiff at the base. As shown in the Figure2 high-frequency sounds localize near the base of the cochlea (near the round and oval windows), while low-frequency sounds localize near the apex.



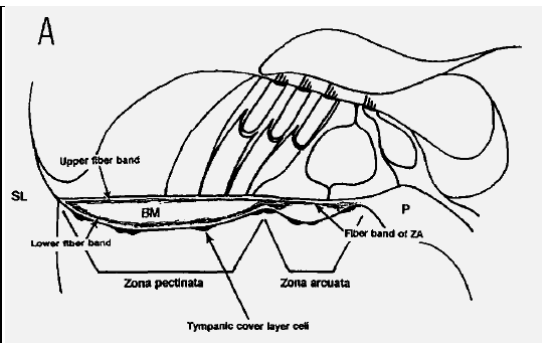
**Figure 1: Position of BM within auditory system**

#### BM zones

The basilar membrane can be subdivided into two zones:(1) the zona arcuata, which underlies the tunnel of Corti and reaches from the osseous spiral lamina to the outer limit of the base of outer pillar cells, and (2) the zonapectinata, which reaches from the outer limit of the outer pillar cells to the spiral ligament (Cabezudo, 1978; Kraus and Aulbach-Kraus, 1981; Rothand Bruns, 1992).



**Figure 2 Cochlea structure**



**Figure 3: Basilar membrane structure with marked regions**

Both zones are comprised of three components - a tympanic cover layer, the ground sub-stance, and one or more fiber bands (Cabezudo, 1978). In all species studied it has been found that within the zonapectinata there are two fiber bands, but within the zonaarcuata only one (guinea pig: Iurato, 1967; cat: Cabezudo, 1978; mouse: Kraus and Aulbach-Kraus, 1981; rat: Rothand Bruns, 1992). The tympanic cover layer consists of epithelial cells on the face of the basement membrane facing the Scala tympani [1].

#### BM radial tension

As shown in the figure 3 the BM can be divided into two regions based on the arrangement of the fibers: The lateral pectinate zone (PZ) where the fibers are grouped into bundles; and the arcuate zone (AZ), where the bundles separate into individual fibers. The parallel arrangement of fiber bundles in the pectinate zone suggests that the bundles are under a radial tension [4]. Studies also suggest that such a tension is maintained by the spiral ligament. It has been shown that the spiral ligament fibers are anchored to the bony cochlear wall by fibroblasts. Fibro blasts contain fibers composed of contractile proteins and have been shown to create tension. In the spiral ligament, the configurations in which fibro blasts are arranged and oriented suggest that these cells actively maintain a radial tension in the BM.

#### BM hair cells

The BM is also the *base* for the sensory cells of hearing, the hair cells that are equipped with "**Stereocilia**". There are approximately 15,000 hair cells in each human ear. Due to its location, the basilar membrane places the hair cells in a

position where they are adjacent to both the endolymph and the perilymph, which is a precondition of hair cell function.

#### Functions of BM

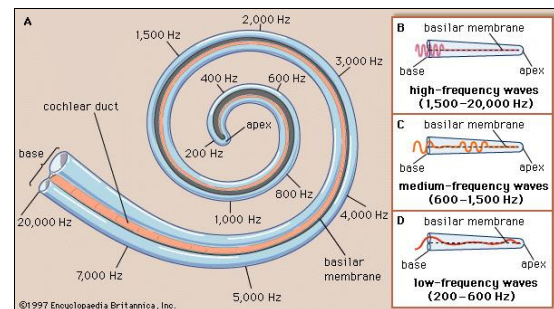
Function of the basilar membrane is strongly developed in the cochlea of most mammalian species i.e. the dispersion of incoming sound waves to separate frequencies spatially. In brief, the membrane is tapered and it is stiffer at one end than at the other. Furthermore, sound waves travelling to the far, "floppier" end of the basilar membrane have to travel through a longer fluid column than sound waves travelling to the nearer, stiffer end.

#### BM model

Each part of the basilar membrane, together with the surrounding fluid, can therefore be thought of as a "mass-spring" system with different resonant properties: high stiffness and low mass, hence high resonant frequencies at the near end, and low stiffness and high mass, hence low resonant frequencies, at the far end. As shown in figure 4 high frequencies lead to maximum vibrations at the basal end of the cochlear coil, where the membrane is narrow and stiff, and low frequencies lead to maximum vibrations at the apical end of the cochlear coil, where the membrane is wider and more compliant.

#### BM tonotopic response

As shown in the figure 4 the "place-frequency map" can be described quantitatively by the Greenwood function and its variants. The Greenwood function is species-dependent and has shown to be preserved in mammals when normalized to the species-dependent range of auditory frequencies and cochlear spiral length



**Figure 4: Tonotopic frequency map of BM.**

For humans, the recommended values for the constants are  $F=165.4(10^{ax} - 1)$ .

According to Greenwood,  $a = 2.1$ , if  $x$  is relative to the cochlea length, and  $a = 0.06$  if  $x$  is calculated in mm.

#### BM mechanics

Sound-driven vibrations travel as waves along this membrane, along which, in humans, lie about 3,500 inner hair cells spaced in a single row. Each cell is attached to a tiny triangular frame. The 'hairs' are minute processes on the end of the cell, which are very sensitive to movement. When the vibration of the membrane rocks the triangular frames, the hairs on the cells are

repeatedly displaced, and that produces streams of corresponding pulses in the nerve fibers, which are transmitted to the auditory pathway. The outer hair cells feedback energy to amplify the traveling wave, by up to 65 dB at some locations

### *BM composition*

Anatomical studies suggest that the basilar membrane (BM) is structurally designed to support a radial tension. As shown in figure 2. BM supports the organ of Corti. The BM is composed of a homogeneous, soft ground substance that is traversed radially by fibers, which extend between the spiral lamina and the spiral ligament. The density of the fiber bundles and fibroblasts decreases from base to apex of the cochlea. The density gradient is accompanied by a visible decrease in the cross-sectional area of the spiral ligament. The variation in fibroblast density, together with the variation in the cross sectional area of the spiral ligament, both suggests that tension, if present, decreases from the cochlear base towards the apex.

### **Section II: BM ANATOMY**

BM within the cochlea is responsible to develop tonotopic frequency map. This ability is attributed to mechanical properties of BM such as mass and stiffness. Mathematical modeling of BM requires precise measurement of BM stiffness. Measurement of various parameters itself is difficult due to accessibility of BM.

Also BM stiffness can be measured in two ways

- a) Hemicochlea preparation
- b) in-vivo

The hemicochlea and its slice preparation is a novel method that allows access to various cochlear structures without the physical distortion that typically occurs from tissue dehydration during the embedding process. Therefore the hemicochlea preparation provides an excellent model to study cochlear morphology during cochlear.

Methods of preparation of hemicochlea are available in the literature (Edge et al. 1998; Hu et al. 1998). We describe typical method for adult gerbil adopted in [6]. Preparation of hemicochlea for adult gerbils can be described as follows

Adult gerbils (*Meriones unguiculatus*) are sedated with chloroform, anesthetized with intraperitoneal sodium pentobarbital (200 mg/kg body weight), killed by rapid cervical dislocation, and decapitated. One bulla is extracted and trimmed to expose the cochlea. The bulla is bathed in modified artificial perilymph, and a sectioning system is used to make a planar cut from apex to base along the modiolar plane of the cochlea. This cut effectively removes one half of the cochlea and leave behind a hemicochlea. The hemicochlea is oriented to yield a cross-sectional view of the tissues in a selected cochlear turn and placed on the stage of an upright microscope, which is located on a vibration isolation table and fitted with a 10X Nikon water immersion objective. The tissue is illuminated off the optical axis using a fiber-optic light guide. Experiments are conducted at room temperature (18°C).

The modified perilymph artificially designed; minimize swelling and deformation of the cochlear tissues relative to their initial state. Reduced calcium, relative to actual perilymph, is necessary and sufficient to maintain the integrity of the tectorial membrane.

Cochlear preparations classified as excellent are only to be included for presentation and analysis. For a hemicochlea to be classified as being in excellent condition, the following morphological features had to be clearly evident: that the tectorial membrane is lifted away from the reticular lamina, the hair cells are not swollen, and the outer pillar cells are not bent.

### *In-vivo*

In-vivo methods of preparation are also described in literature for example in [2]

We typically describe method followed in [6]

In vivo stiffness measurements are obtained from the cochlea of adult gerbils (*Meriones unguiculatus*, >60 days after birth) of either sex. Each gerbil is anesthetized by intraperitoneal sodium pentobarbital (80 mg/kg body weight). Maintenance doses of pentobarbital (17 mg/kg body weight) are given whenever the animal shows signs of increasing arousal, as assessed by a paw withdrawal reflex. After the animal is fully anesthetized, breathing is facilitated by inserting a short length of tubing into the trachea, and body temperature is maintained at 38°C using a heating pad. The animal is positioned on its back, and its head is stabilized in a heated head holder. The right submandibular gland is exposed by making an incision from the lower right jaw to the right shoulder. The gland is then ligated and removed to reveal the muscles attached to the bulla and to the styloid bone. The muscles are dissected away to expose the portion of the stapedial artery at the medial plane of the bulla. To minimize the risk of bleeding during further manipulations, the artery is tied off at 2 positions as close as possible to the bulla and is cut between the knots. The bulla is then opened to allow access to the cochlea.

Before opening the cochlea itself, an electrophysiological assessment of its function is made, as follows.

A silver electrode is hooked onto the bony rim of the round window, and a ground electrode is placed under the skin at the left jaw. The cartilaginous portion of the external auditory meatus is removed, and a brass speculum is cemented with dental acrylic to the bony portion of the external meatus. The animal is then moved onto a vibration isolation table in a soundproof booth, and a high-frequency tweeter is coupled to the speculum at the ear canal. To document baseline hearing function, an auditory nerve compound action potential (CAP) threshold curve is measured using a modified tracking procedure (Gummer et al. 1987; Taylor and Creelman 1967). The CAP is generated by synchronized activity in the auditory nerve, and the CAP threshold measured at a particular frequency using narrow-band stimulation is an indicator of cochlear function localized to the characteristic place for the measurement frequency. CAP thresholds are measured over a 5-octave range between 1.6 and 50 kHz, with a resolution of 6 steps per octave. The stimuli are tone bursts lasting 10 ms



(ramped at 1 ms), and the threshold at a given frequency is defined as the sound level required to generate 10  $\mu$ V N1-P1 amplitude in the CAP waveform. The contribution of cochlear microphonics is reduced by averaging responses over 32 consecutive tone burst presentations delivered in pairs of opposite phase. It typically takes about 20 min to obtain a single CAP threshold curve with the range and resolution described above.

After determining the baseline CAP thresholds, the casing around the portion of the stapedial artery overlying the cochlea is removed during a sharp Weaver blade, and the artery is gently flipped out of the way.

To expose the BM, an opening is scored in the bone over scala tympani in the basal turn of the cochlea at a location ranging 2.0 to 2.8 mm from the base (characteristic frequencies: 17.3–25.1 kHz based on Muller 1996). The opening is approximately rectangular, with a length of  $\leq 500$   $\mu$ m along the cochlear spiral and a width of  $\leq 100$   $\mu$ m.

To determine whether any damage occurred during the opening of the cochlea, CAP threshold curves spanning the entire frequency range are obtained at intermediate stages of the procedure. BM stiffness is measured. A final CAP-threshold curve is obtained to document any changes in hearing function caused by contacting the BM.

The essential differences between a hemicochlea and the in vivo cochlea preparation are as follows: 1) the hemicochlea is removed from the living animal; 2) the hemicochlea entails a gross cut through the tissue, whereas the in vivo cochlea is intact except for a small opening in the lateral wall over scala tympani; and 3) in the hemicochlea all of the fluid spaces are breached and filled with a perilymph-like solution, whereas in vivo scala media is filled with endolymph. One important consequence of these differences is a significant alteration of the electrical environment that surrounds the cochlear hair cells.

#### Shift of the cochlear place code

As the cochlea develops, the cells in the basal cochlea become sensitive to progressively higher frequencies (Lippe and Rubel, 1983; Rubel and Ryals 1983). It is generally accepted that as the cochlea develops, neural elements in the basal-most turns are initially more sensitive to low frequencies and later become more sensitive to progressively higher frequencies. This is commonly called the 'shift of the cochlear place code'.

Figure 5 shows frequency coding in the adult gerbil cochlea and the predicted frequency representation in the post natal day PND13 gerbil. Frequency is represented on the top two scales; place is represented on the bottom scale. For the present study two kinds of comparisons were made and these are represented by the vertical and diagonal arrows.

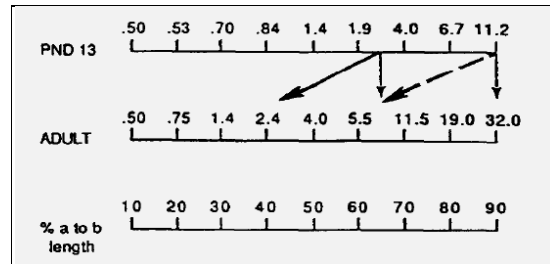


Figure 5 Place code change in PND and adult gerbil

Vertical arrows indicate cross-age comparisons of the same cochlear location. Diagonal arrows indicate comparisons of two regions thought to code for the same frequency at two different times in development. 11.2 kHz (dashed arrow) is first represented at about 90% of the apical to basal (% a to b) distance but later at 65% of the apical to basal distance and 3 kHz (solid diagonal arrow) is predicted to shift from 65% to 42% of the apical to basal distance [1].

Data on frequency place code shifts in the bat *Rhinolophus rouxi* also show that there is an addition of high-frequency tips to low-frequency tails during development (Rubsamen and Schafer, 1990). Indeed, BM morphology including the thickness of the fiber bands in the BM change gradually after the onset of cochlear function and during the preweaning period when the place code is shifting (Weaver, 1993). Other evidence supports the notion that 'active' cochlear mechanics are relatively mature just after the onset of cochlear function in the gerbil. Woolf and Ryan (1985) found that the tuning curves of high-frequency cochlear nucleus cells at PND 15 were as sharp as adult curves. That 'active' OHC-related processes are mature at this time is supported by the presence of the action cytoskeleton (Romand et al., 1993; Weaver et al., 1994) and electromotile properties (He et al., 1994) in OHCs and by the adult-like cochlear amplifier gain for  $f_c$  frequencies from 2 to 8 kHz (Mills et al., 1994). These indices of maturity are achieved at least in the basal and middle gerbilline cochlea before the shift of the place code takes place. This suggests that ongoing maturation of 'passive' cochlear mechanics contributes to the shift of the frequency place map for these frequencies.

The thicknesses of the upper and lower fiber bands in the BM change in a way that is consistent with the place code shift [1].

#### BM anatomical changes

Measure	PND 12/13			
	2%	42%	65%	90%
Duct area *		65493	59999	103743
BM area *		2468	1926	1492
BM width **		239.4	182.3	159.4
BM height **		23.1	19.9	18.1
TCL thickness **		14.1	16.1	21.8
BM up band **	0.136	0.287	0.395	1.160
BM low band **	0.209	0.489	0.833	1.161
Organ of Corti area *	9996	9038	6192	5359
BM op dens ***		155.3	154.0	145.0

Table 1 BM variable anatomical changes

As shown in the table above during the development of BM it exhibits changes in various physiological parameters [1]. Figures 5,6,7 and 8 shows the BM anatomical changes [1].

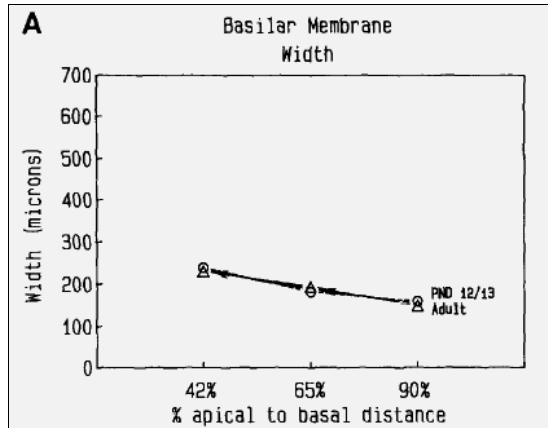


Figure 6 BM width variations

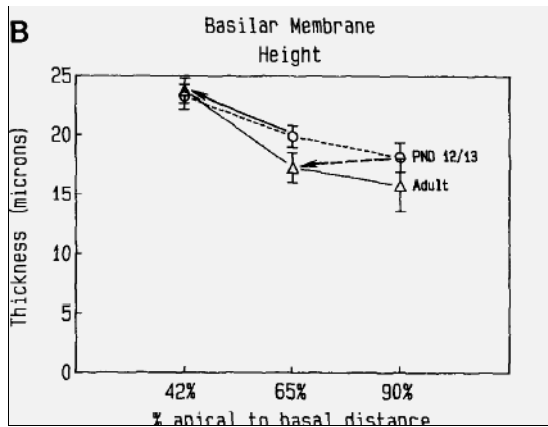


Figure 7 BM height variations in PND and adult

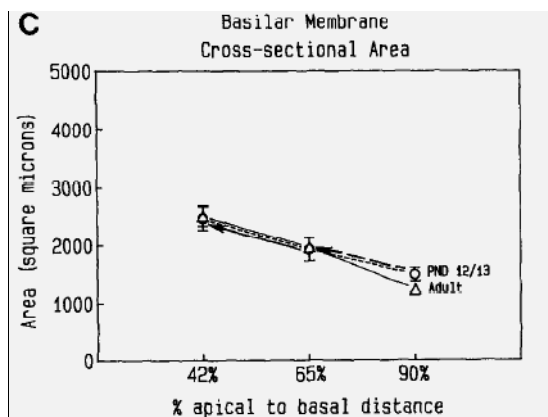


Figure 8 BM Cross section area variations in PND and adult

### SECTION III BM SIGNIFICANCE

#### Cochlear and BM model equation

BM stiffness dominates the tonotopic response of the cochlea [7] as suggested by various mathematical models for cochlea

as well as BM. Mathematical modeling of cochlea is available in literature (Stephan Neely, 1977,) Typical BM and cochlear parameters are listed in table 2[7]. Figure 9 and 10 depicts the amplitude and phase response of BM [7]. Model equation for BM is given as below [7]

$$\frac{k \tanh(kh)}{1 + \alpha S(x) k \tanh(kh) (\gamma e^{ikd} - e^{-ikd}) / (2\rho\omega^2)} = \frac{2\rho\omega^2}{S(x) + i\omega\beta(x) + (i\omega)^2 M(x)}$$

Equation depicts the factor  $S(x)$  on the right hand side which is the stiffness which is more significant than the mass of BM depicted in the equation as  $M(x)$ . Hence BM stiffness measurements are essential to explain the properties of BM and its tonotopic response.

Parameters and denotation	Values	Unit
Cochlear duct height $h$	1.0	mm
Cochlear duct length $L$	25.0	mm
Fluid density $\rho$	$1.0 \times 10^{-3}$	$\text{g}/\text{mm}^3$
BM mass per unit area $M(x)$	$3.0 \times 10^{-5}$	$\text{g}/\text{mm}^2$
BM stiffness per unit area $S(x)$	$5.0 \times 10^6 e^{-0.4x}$	$\text{g}/(\text{mm}^2 \text{s}^2)$
BM damping ratio $\zeta$	0.2	
Tilt distance $d$	$71.0 \times 10^{-3}$	mm
Segment length $\Delta$	$1.0 \times 10^{-2}$	mm
OHC motility factor $\alpha$	0.0 - 0.2	
Forward-to-backward ratio $\gamma$	0.3	

Table 2 Cochlear and BM parameters

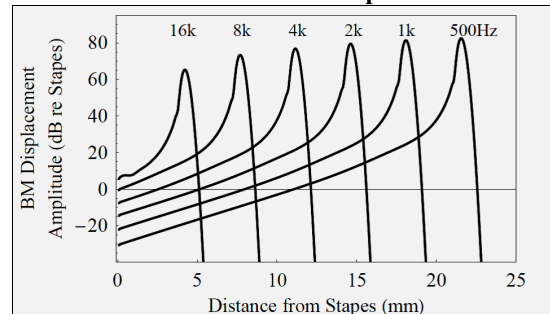


Figure 9 BM amplitude response

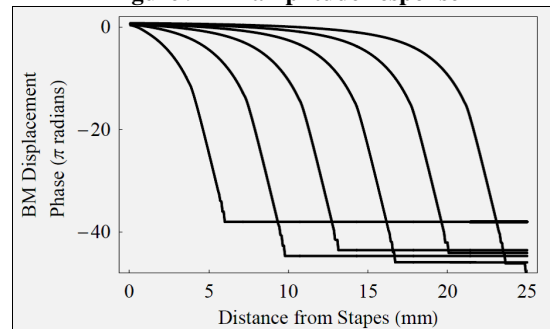


Figure 10 BM phase response

## II. FUTURE SCOPE AND DIRECTION

Various cochlear models have been presented in literature. A mathematical model equation involves BM stiffness as dominating factor. BM mechanics alteration can bring out changes in response of hearing. Various factors can bring about BM response. Our focus is to investigate the stiffness change due to thickness changes introduced due to diabetic condition.

Prolonged hyperglycemic condition leads to thickening of vessels of BM which means progressively changing  $M(x)$  and  $S(x)$  making them time variant. It means that the model parameters  $M(x)$  and  $S(x)$  which are space variant will also be made time variant. Due to overall stiffness change the BM may not be able to peak at the same amplitude level [8].

### CONCLUSION

BM within cochlea plays a central role in auditory mechanical transduction. BM shows variation in its mechanical properties during its earlier stage of development which results into tonotopic shift. Measurement of stiffness of BM is very essential in order to predict the response of mathematical model of cochlea. It can be measured in various ways hemicochlea or in-vivo. Changes in BM mechanical properties can change the tonotopic response and result in hearing impairment.

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