Sound-Induced Micromechanical Motions in an Isolated Cochlea Preparation

by

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Abstract:

The mechanical processes at work within the organ of Corti can be greatly elucidated by measuring both radial motions and traveling-wave behavior of structures within this organ in response to sound stimuli. To enable such measurements, we have developed a new preparation for observing three-dimensional motions of micromechanical structures in the apical region of an isolated gerbil cochlea. The cochlea is submerged in a low-chloride, low-calcium artificial perilymph solution and cemented to the bottom of a Petri dish at an angle. The bone above scala vestibuli of one half of the apical turn is removed to allow optical imaging with a 40x, 0.8 NA water-immersion objective. Reissner's membrane is left intact. Illumination is provided with a blue LED coupled to an optical fiber. The fiber is positioned next to the bone surrounding scala tympani of the apical turn, so that the organ of Corti is illuminated from below. The resulting optical access allows imaging of a variety of structures that have been proposed to play a role in cochlear mechanics, including inner and outer hair cell bundles, the tectorial membrane, inner and outer pillar cells, and efferent fibers in the tunnel of Corti. In some preparations, individual stereocilia of inner hair cell bundles can be resolved. Motions are stimulated by driving the stapes with a piezoelectric probe, and are measured using a stroboscopic computer microvision system. Measurements of sub-micrometer motions of key structures in three dimensions are quantified, including longitudinal motion of the organ of Corti and relative radial motion between the tectorial membrane and hair cells. Longitudinal motion of the Efferent fibers in the tunnel of Corti is found to have a phase lead with respect to the hair cell bodies. This system enables quantitative studies of both the relative motions of structures within the organ of Corti in response to sound and the propagation of traveling waves along structures within the organ of Corti.

Thesis Supervisor: Dennis Freeman
Title: Associate Professor

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This is a big accomplishment for me and I am very thankful to those who have helped me come this far. I'd like to thank Dennis Freeman for allowing me to work with him and being a great advisor. He has a lot going on, seemingly every day of the week, but would routinely take time out to discuss research progress. In addition to academic discussions, I have enjoyed our social discussions, sometimes political, which has made working with him a lot of fun.

I'd like to thank the following lab mates who have become good friends to me as well:

A.J. has mentored me throughout this project and is extremely patient at times. I especially enjoyed sharing the excitement of the results as they were discovered, cause when A.J. gets excited about a result, it's a good indicator that the work is going well. He is very enthusiastic and a lot of fun to work with, and he holds all the jellybeans so he's fun to visit.

Rooz helped me learn the ropes of the lab, including my first mouse, and perilymph batch. He was always interested in discussing experiments with me, which is a great help. I enjoyed playing basketball with him, and seeing him apply those skills to throwing pillows at my head.

Stan helped me a lot on my quals and is a great officemate and researcher. He also taught me to golf, which I hope to put to use some day. Hopefully, in Pebble Beach when I visit him at his California mansion next year.

Chris frequently asks how things are going, and many times this innocent question develops into a full-blown whiteboard session with extremely useful ideas. I sincerely appreciate that. Snowboarding was great, we went big, but lets avoid the mogul rock alley next time.

Wendy also helped me with getting to know the lab, and set me up with a piezoelectric crystal. She has an extremely impressive memory and brought the group together with her barbeques and cakes.

Kinu taught me a lot about her work on the tectorial membrane. She has a lot of energy and contagious excitement that keeps the research morale high. If research morale were a half-wave rectified signal, Kinu would be a large capacitor.

Salil is a lot of fun as an officemate. He is a man with a plan, and has an important sounding name. He gave me extremely good advice for my quals and even let me borrow a cup of KOH once.

I'd like to thank Gary and Peggy, for being great mentors. I'd also like to thank my brother Randy, and my friends Anthony, Cheyne, Dave, Ian, Isaiah, Mikey, Ryan, and Troy for your support throughout the years, and for encouraging me to go to college.

Most importantly, I'd like to thank my girlfriend Sonia for her love and support during these sometimes stressful times at MIT. She has helped considerably with her patience and her ideas about the progress of the work, after my tireless rantings each night.

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Chapter 1

Introduction

1.1 The Mammalian Cochlea

Beneath the reticular lamina and supported by the basilar membrane, the organ of Corti consists of a variety of cells including inner and outer hair cells, which are separated radially by pillar cells, supporting cells, and the tunnel of Corti. Each inner and outer hair cell projects stereocilia into the sub-tectorial space, which lies above the reticular lamina and beneath the gelatinous, acellular structure called the tectorial membrane (Freeman et al, 2003). There, it is generally believed that the stereocilia of the outer hair cells come into contact with the tectorial membrane (TM). Micromechanical measurements of these interconnected cochlear structures are essential to understanding the overall behavior of the mammalian cochlea.

The cochlea, which is part of the mammalian inner ear, consists of three fluid filled cavities: scala vestibuli, scala media, and scala tympani. Scala vestibuli and scala tympani are filled with perilymph (a high sodium, low potassium fluid) and are connected by the helicotrema. The scala media, filled with endolymph (a high potassium, low

sodium fluid) sits between them and is separated from scala vestibuli by Reissner's membrane, a thin layer of epithelial cells, and from scala tympani by the reticular lamina, a system of tight junctions along the surface of the organ of Corti.

Sound travels into the cochlea via the tympanic membrane, which excites the three middle ear ossicles: the incus, malleus and stapes. The stapes is coupled to the scala vestibuli at the oval window, and provides the final step of impedance matching between the air-filled middle ear and the fluid filled cochlea. As the stapes is displaced, a traveling wave of pressure differentials between the scala vestibuli and scala tympani travels from the base of the cochlea towards the apex. This macromechanical pressure wave causes a displacement of the basilar membrane and organ of Corti. This displacement is maximal toward the base for high frequency stimuli and toward the apex for low frequency stimuli.

As technology advances and improvements to cochlear mechanical measurements are made, our understanding of how the cochlea works is increased. Issues such as the invasiveness of a measurement technique, the type of stimulus used, whether the experiment is conducted *in vivo* or *in vitro*, and the resolution and the dimensional scope of the measurements can greatly influence the conclusions reached.

1.2 Previous Research

The measurement of absolute and relative motions of cochlear structures under a variety of stimuli is an active area of investigation in the hearing sciences. In the early

part of the twentieth century, Georg von Békésy (von Békésy, 1960) made important observations in cochlear mechanics. Using stroboscopic light microscopy to visualize the response to very large stimuli (> 120 dB SPL) in the apex of cochleae from cadavers, von Békésy was able to estimate the motion of the basilar membrane as a traveling wave propagated along the cochlear partition. From these observations he concluded that the amplitude of the motion of the basilar membrane increased linearly with the input sound pressure.

Johnstone and Boyle (Johnstone et al, 1967) quantified the basilar membrane motion observed by von Békésy using the Mössbauer technique. The Doppler shift in photon frequency of a radioactive source (0.008 mm^2) placed onto the basilar membrane was measured with a photon absorbent material in response to tones. This technique allowed for basal areas of the cochlea, which are more sensitive to high frequency inputs, to be measured. Johnstone and Boyle, using dead cochleae, again found a linear basilar membrane response to sound pressure, but with a steep high frequency slope.

Using the Mössbauer technique in live animals, Rhode (Rhode, 1971) was able to see extensive nonlinearities at the peak of the curve as sound pressure was reduced down to a resolution limit of approximately 70 dB SPL. Ruggero and Rich (Ruggero et al, 1991) quantified basilar membrane motion in live cochleae using the more sensitive laser velocimetry technique. In this method, a glass bead was placed onto the basilar membrane, and the Doppler shift of the reflection of laser light focused onto the bead was measured. Ruggero and Rich found nonlinear basilar membrane motion in response to sound, a higher sensitivity to low sound pressures than previously reported, and sharper tuning at the characteristic place.

These results contributed to the theory of an active cochlear amplifier, in which an increase in the response of the traveling wave to low sound pressures occurs through the electromotile length changes of outer hair cells in response to varying membrane potentials (Brownell et al, 1985). Modelers have analyzed cochlear mechanical measurements and physiological data to try to understand how and where this amplification is taking place.

From cochlear models based on laser velocimetry measurements of the basilar membrane by Nuttall, de Boer (de Boer, 1996) found that the response of the basilar membrane was dominated by stiffness at frequencies below its best frequency, and by mass at frequencies above its best frequency. The impedance of the basilar membrane was found to be negative at a place below the best frequency, suggesting an addition of energy at that place possibly by outer hair cells. Insight into how this additional energy on the basilar membrane is related to the motion of outer hair cells can be gained by the measurement of structures along the reticular lamina, the tectorial membrane, and within the Organ of Corti.

Khanna (Khanna et al, 1999) developed a measurement system using laser interferometry coupled into a slit confocal microscope. With this system, they looked at the transverse micromechanical motions of structures along the reticular lamina of an exposed apical turn of an *in vivo* preparation in response to sound. An advantage of the confocal interferometry method was that Reissner's membrane could be kept intact because there was no need for a target bead.

Gummer (Gummer et al, 1996) used a combination of laser interferometry and a photodiode to study two dimensions of motion (radial and transverse) of the tectorial

membrane in an *in vitro* cochlear preparation in response to sound. Care was taken to maintain the air space in the middle ear. In 2000, a differential photodiode technique in combination with laser interferometry enabled three dimensions of reticular lamina and basilar membrane motion to be measured (Hemmert et al, 2000). These experiments demonstrated tectorial membrane resonance, an important feature of many cochlear models.

Chan and Hudspeth (Chan et al, 2005) isolated the second turn of the gerbil cochlea and stimulated it both acoustically and electrically. Acoustic stimulation was provided by placing the isolated turn into a chamber and delivering sound via a tube. Motions of the basilar and tectorial membranes were measured in the transverse direction with laser interferometry using glass beads and in the radial and longitudinal directions with a photodiode projection system. As another form of measurement, stroboscopic video microscopy was used to take images of the motion of structures throughout the cochlear partition, which was then quantified using an optical flow algorithm. A substantial radial motion was seen at the reticular lamina. Additionally, a difference in radial motion of structures near the reticular lamina was seen when stimulated electrically versus acoustically.

Ulfendahl (Fridberger et al, 2006) measured the motion of stereocilia, in response to sound, in an *in vitro* cochlear preparation using time-resolved confocal imaging. Motions were quantified using a wavelet based optical flow algorithm. This technique allows good resolution of structures such as stereocilia, but not the tectorial membrane. Additionally, although this technique can be used to measure motions in three dimensions, the necessity to scan point by point makes it very time cumbersome.

1.3 Contributions of this Research

Relatively little is known about the relationship between input sound stimuli, macromechanical motions (such as basilar membrane motion), and the micromechanical motions of the tectorial membrane and structures within the organ of Corti. This thesis work is focused on the imaging and measurement of relative three-dimensional micromechanical motions of cochlear structures, including the inner and outer hair cell bundles, the tectorial membrane, and other visible structures. An *in vitro* nearly intact isolated cochlea preparation is stimulated with an acoustic stimulus produced by displacing the stapes. Although a small viewing hole is made in the bone of the apex, Reissner's membrane is left intact to preserve the fluid separation between scala vestibuli and scala media. Micromechanical measurements are obtained and quantified using optical flow algorithms.

This work adds to the field by providing three-dimensional micromechanical motion measurements of structures throughout the cochlear partition and also the tectorial membrane down to approximately 40 nm resolution. It does so using a nearly intact isolated cochlea with an acoustic stimulus. This method currently allows the passive mechanics of the cochlea to be investigated, and has the potential to be applied to *in vivo* preparations to investigate the active cochlea. The preparation may also be used to investigate issues such as the number of modes of micromechanical motion of the organ of Corti, the extent of tectorial membrane resonance, the tuning of the basilar membrane with regard to inner hair cell motion, and how longitudinal motion of the organ of Corti affects hearing.

Chapter 2

Methods

This chapter details the preliminary work on mouse cochleae and experimental work on gerbil cochleae.

2.1 Stimuli delivery system

The isolated cochlea was stimulated via stapes displacement. A stimulus delivery system was designed and constructed for this task using a mechanically loaded piezoelectric crystal with an attached metal probe tip.

2.1.1 Prototype Design

The prototype design consisted of a wide base plate upon which one end of a steel tang was attached via a riser. The piezo was epoxied to a plastic base, which was raised along 3 guidance screws using an adjustment screw. The base was raised into position so that the piezoelectric crystal was flush and in tight contact with the steel tang. Varying

sinusoidal voltages, with a larger amplitude DC offset, were applied to the piezo and the motion of the steel tang was measured using a laser Doppler interferometer. A signal analyzer was used to analyze the frequency response of the prototype, which was determined to have its first resonant peak at approximately 400 Hz, and a linear phase response up to 6 kHz.

2.1.2 Final Design

Based on the relative success of the prototype, another version of the stimulus probe was constructed with the goal of making the design more compact, with a higher frequency range. In this design, shown in figure 2-1, a much smaller base was used with screws and nuts tightly holding the thinner and smaller tang. The piezo was directly mounted and raised against the tang using a set screw. Measurements using the signal analyzer gave the first resonant peak at 446.7 Hz and a linear phase response up to approximately 12 kHz.

The motion of various probe tips of varying lengths affixed to the end of the steel tang was analyzed using the stroboscopic light microscopy system. Blue LED light was strobed onto the water immersed probe tips using an optical fiber and pictures taken at various phases of the delivered sinusoidal voltage. Figure 2-2 shows a sample image of the probe tip at 40x magnification. This method allowed for video images of the motion of the probe tip to be made at various frequencies and amplitudes. Figure 2-3 shows that the probe tip displacement versus input piezo voltage is roughly linear. It was determined

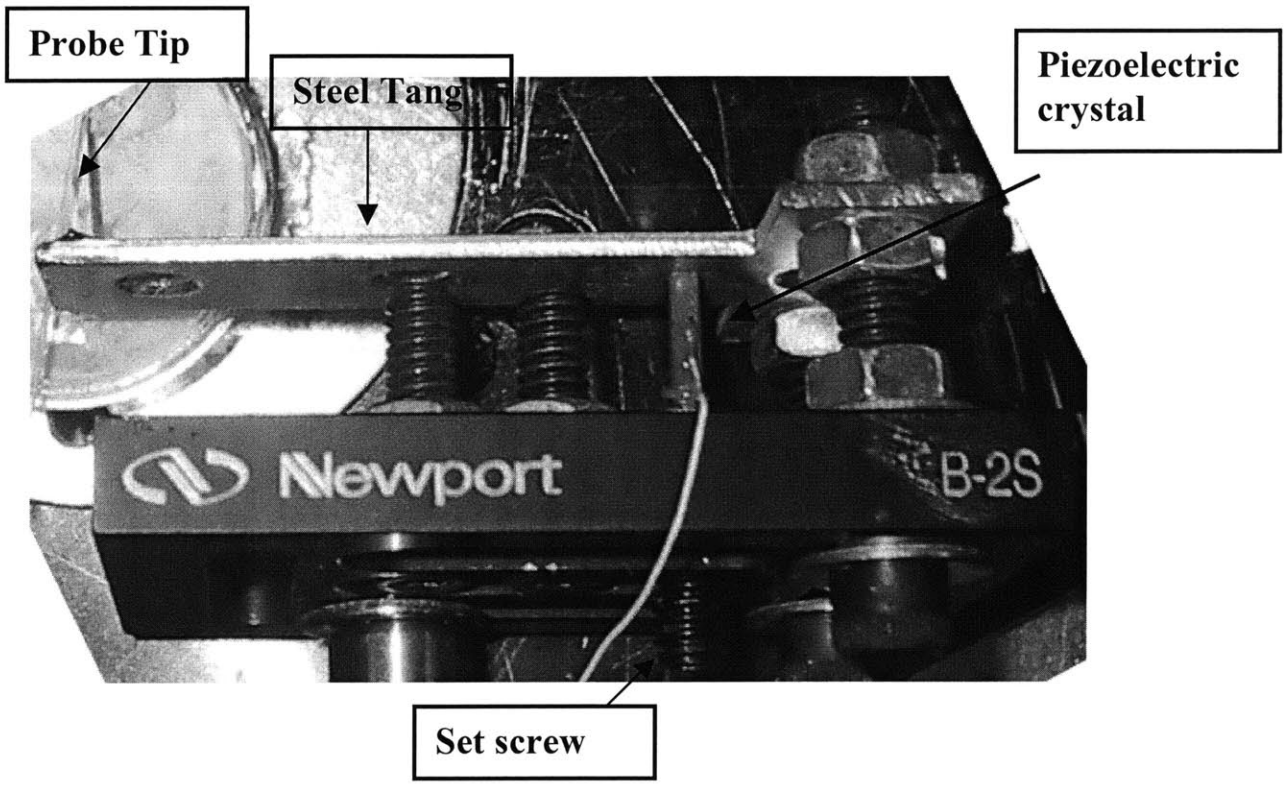


Figure 2-1: Stimulator probe. A piezoelectric crystal is mounted on a base, attached to a micromanipulator, and loaded by a steel tang. The probe tip can then be maneuvered into position and used to deliver sinusoidal displacements

that a short titanium tip was ideal to maximize the frequency delivery capabilities of the stimulus delivery system.

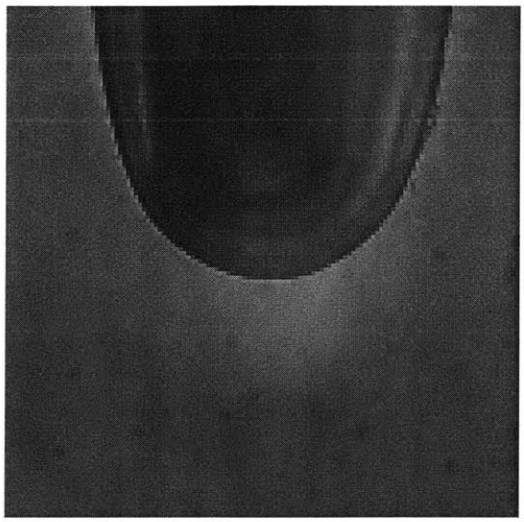


Figure 2-2: Probe tip immersed in water at 40x magnification. This is one frame of a sequence of stroboscopic images taken while determining the tip displacement versus input piezo voltage.

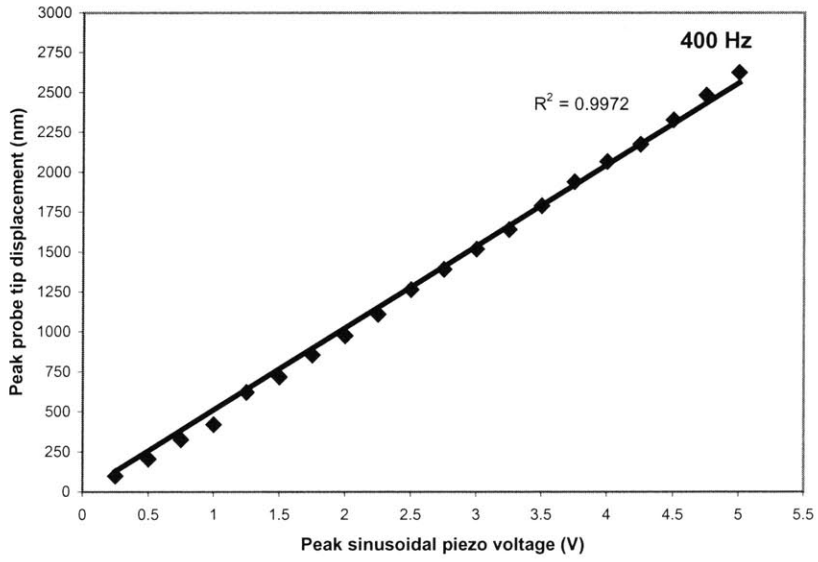


Figure 2-3: Probe tip displacement versus input piezo voltage at 400 Hz. Sinusoidal voltage applied to the piezoelectric crystal resulted in a sinusoidal probe tip displacement.

2.2 Cochlear Preparation

2.2.1 Preliminary Preparation

A preliminary determination of the visual clarity of cochlear structures was accomplished using light microscopy. Mice were used to determine whether structures would be visible using the proposed light microscopy techniques. The mice were euthanized with CO_2 and decapitated. Exploratory surgeries were attempted medially from the outer ear canal in an effort to isolate the cochlea while preserving the stapes, round window, and integrity of the cochlea. This entire procedure was later repeated with Mongolian gerbils (*meriones unguiculatus*) and the results for these sections are shown.

2.2.2 Artificial Perilymph

The cochleae were isolated in a bath of low Ca, low Cl, artificial perilymph consisting of 7 mM sodium chloride (NaCl), 163.4 mM sodium gluconate, 3 mM potassium chloride (KCl), 0.1 mM calcium chloride dihydrate ($CaCl_2 \bullet 2H_2O$), 0.1 mM magnesium chloride ($MgCl_2$), 2.0 mM sodium sulfate (Na_2SO_4), 0.5 mM sodium dihydrogen phosphate (NaH_2PO_4), 5 mM HEPES, 5 mM dextrose, 4 mM L-glutamine. The artificial perilymph was brought to a pH of 7.30 using potassium hydroxide (KOH).

2.2.3 Surgical Methods

With the goal of making sound-induced measurements of cochlear structures in the Mongolian gerbil, careful efforts to preserve the stapes during cochlear isolation were made. Mongolian gerbils were euthanized with CO_2 and decapitated. The tissue surrounding the bulla was removed to facilitate precise removal of the bulla without cochlear damage. Figure 2-4 shows a view of the middle ear space and cochlea inside the bulla during cochlear isolation. Precise cuts to the stapedius and tensor tympani tendons allowed the stapes to be safely separated from the incus, so that the middle ear could be pulled away. The cochlea was then isolated through removal of surrounding supporting bone, while preserving the vestibular canals, round window, and integrity of the cochlea.

Two-part dental cement (Durelon), with a working time of two and a half minutes, was mixed on the bottom of a dry Petri dish. Excess, unmixed dental cement was then rinsed away with deionized water and the Petri dish was filled with artificial perilymph. The isolated cochlea was placed apex-up in the dental cement and allowed to harden in place as diagramed in figure 2-5. Using a number 12 scalpel, a small rectangular hole was scored into the apical bone. The bone was then penetrated and force exerted from the bottom of the rectangle upward to pop out the scored section.

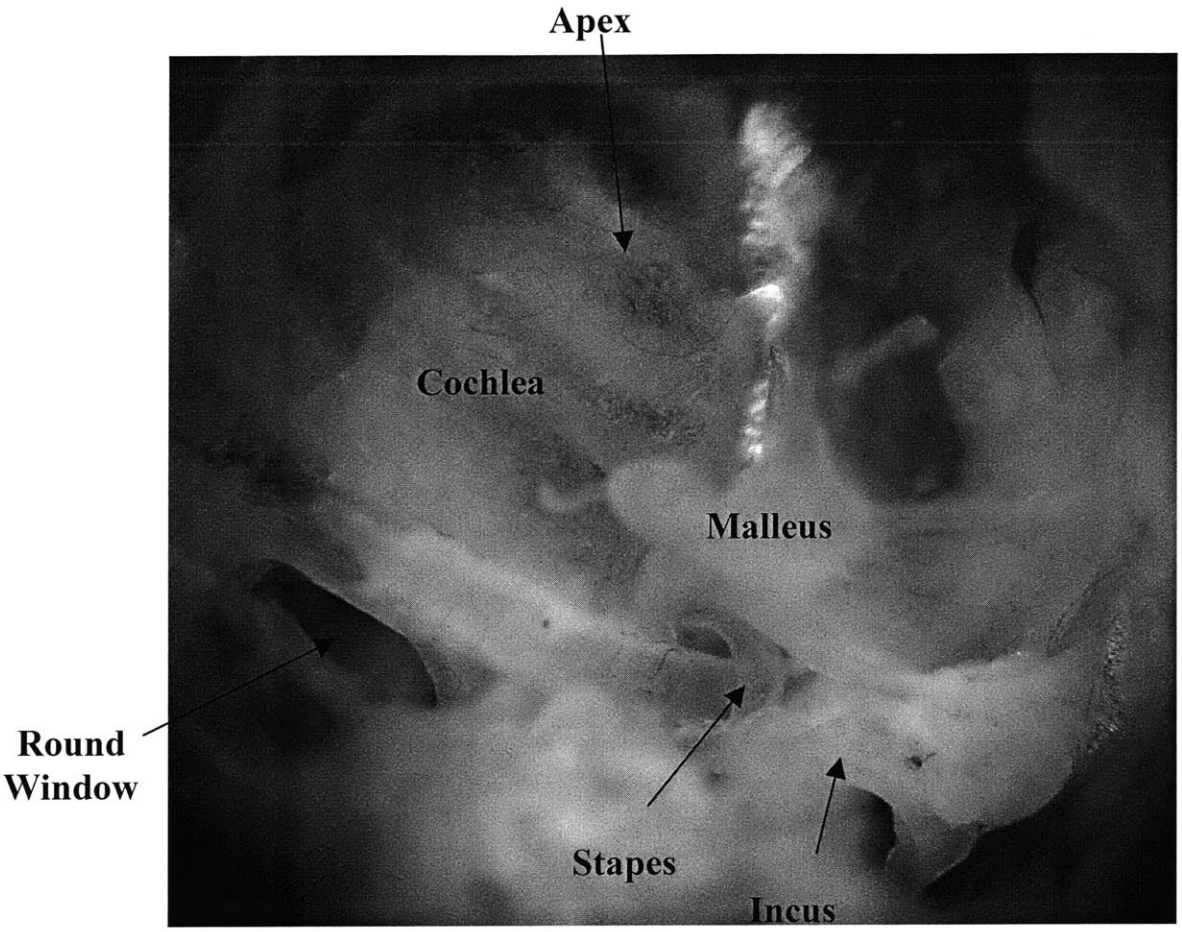


Figure 2-4: View of mammalian middle ear space and cochlea during cochlear isolation. The three ossicles of the middle ear are visible, as well as the round window (base) and apex of the cochlea.

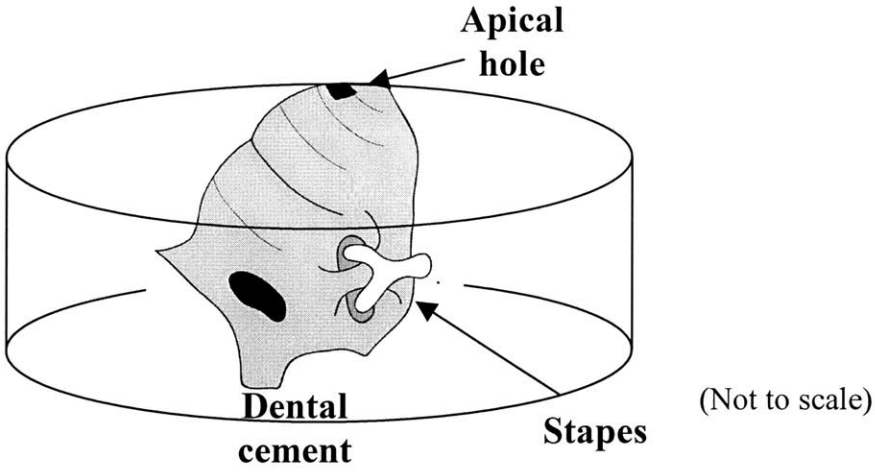


Figure 2-5: Diagram of cochlea cemented upright in Petri dish. The entire cochlea is submerged in perilymph and a hole has been made in the apex for optical access.

This procedure was repeated frequently to develop knowledge and surgical speed during cochlear isolation, to develop the apical cut method, and to determine the angle at which the cochlea must be mounted and the position and size of the hole needed in order to successfully view structures at 40x with Reissner's membrane intact.

2.3 Optical Setup

The cochlea was then placed on the stage of a light microscope (Zeiss Axioplan, Thornwood, NY), which was mounted on a pneumatic vibration-isolation table. A blue LED coupled to an optical fiber was used to illuminate the apical portion of the cochlea. As detailed in figure 2-6, the apical hole was centered in the microscope field of view and the optical fiber was aligned using a 5x objective. Using the 5x objective the stimulator probe tip was guided into contact with the head of the stapes. Cochlear structures were

brought into focus under a 40x water immersion objective (0.80 NA) and adjustments made to the illumination by changing the position of the optical fiber. A view of the external setup is shown in figure 2-7.

A 12-bit CCD camera (Diagnostic Instruments, Sterling Heights, MI) with an array of 1024 by 1024 pixels was coupled to the microscope to capture stroboscopic images. Fig 2-8 shows the contrast between a raw image of the organ of Corti taken at 40x magnification, and the same image with the dynamic range adjusted. The bit depth of the camera allows many cochlear structures to be visualized by adjusting the dynamic range of the input to maximize contrast differences as illustrated in section 3.1.

2.4 Experimental Parameters

Sinusoidal voltages were applied to the stimulator at various amplitudes ranging from 0.1 V to 5 V (corresponding to approximately 85 dB SPL to 130 dB SPL) and frequencies ranging from 50 Hz to 450 Hz. For each stimulus amplitude and frequency measurement, a sequence of images was collected at micron spaced focal planes over a 100 μm depth. Each sequence consisted of eight images, corresponding to eight evenly spaced phases of the stimulus cycle. Focal depth was controlled using a piezoelectric microscope focusing system (Physik Instruments). Stimuli levels were increased as needed, and after each 100 μm run a control experiment was done (See section 2.6). Generally, each 100 μm set of images took an average time of about 45 minutes to complete.

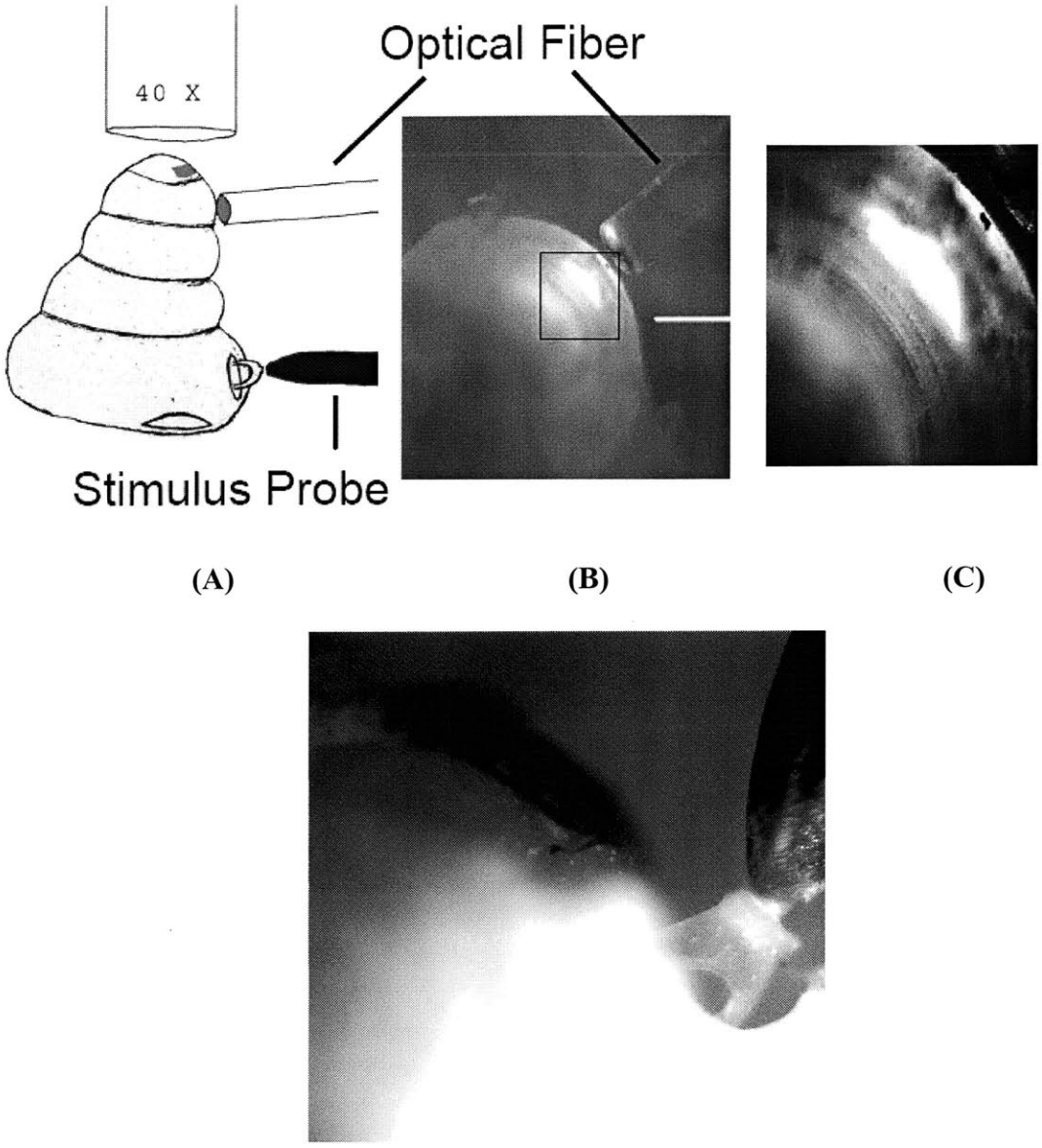


Figure 2-6: Optical fiber illumination and stimulator probe alignment. (A) Diagram of the isolated mammalian cochlea under the microscope. (B) View of the apex through the microscope at 5x magnification. The boxed area shows the apical hole, which the optical fiber is illuminating. (C) Inset: Image of the organ of Corti through the apical viewing hole. (D) Probe tip contacting head of stapes at optimal angle.

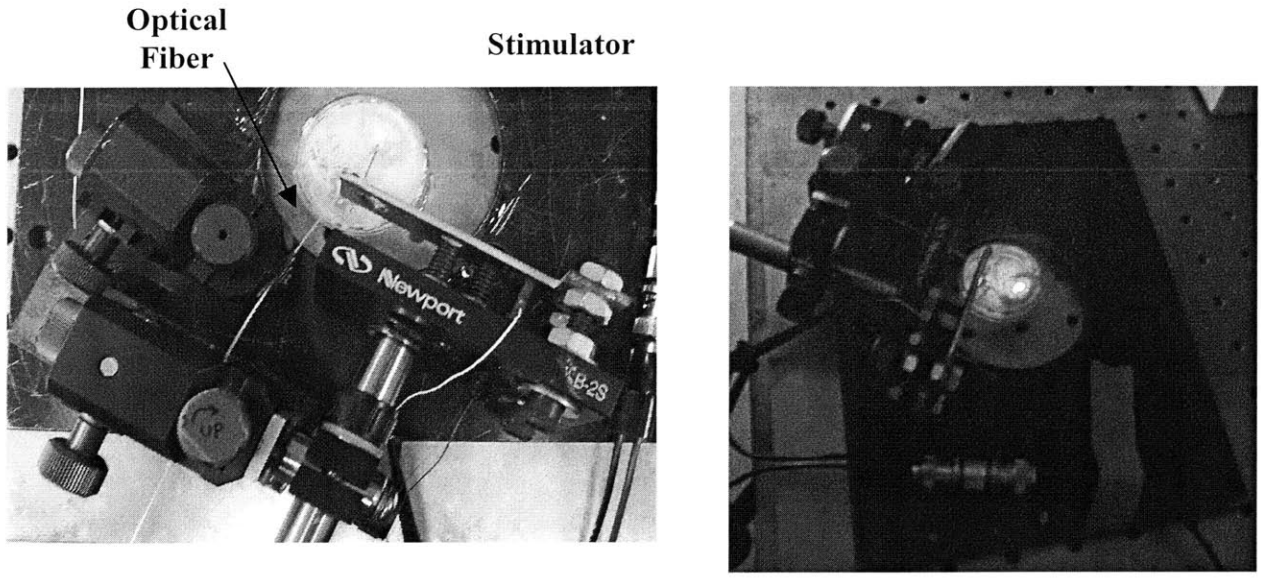


Figure 2-7: Experimental setup. (A) Magnetically mounted micromanipulators align the optical fiber and stimulator probe with cochlea. The cochlea is mounted to the Petri dish in dental cement and the dish epoxied to the base plate. (B) View of the setup during stroboscopic illumination.

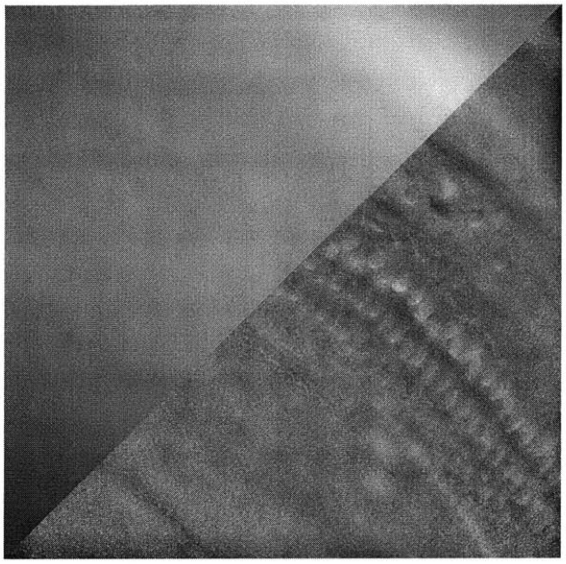


Figure 2-8: Contrast between a raw image and the same image with dynamic range narrowed. The image is of an apical turn of the organ of Corti taken at 40x magnification. (Upper-left) Raw image. (Lower-Right) Image with adjusted dynamic range. The cochlear structures visible are outlined in figure 3-1.

2.5 Motion Measurements

2.5.1 Algorithms for Motion Measurements

Optical flow algorithms which track brightness gradients were used to analyze the three dimensional motion of identified cochlear structures. The algorithm returns the estimated amplitude and phase of the sinusoidal motion for a specified region of interest. To verify the motion measurements, scripts were developed to move each image by the estimated amplitude in the opposite direction. If the motion measurements were correct, the sequence of images would appear to stand still.

Generally, the estimations of motion produced by the optical flow algorithm did not precisely measure the motion on the first attempt. Scripts were developed to recursively submit the result of the reversed amplitude estimations into the optical flow algorithm, and iteratively sum the estimations until the motion of the structure of interest in the reversed motion image was minimized. See Appendix.

2.5.2 Motion Analysis

The motion of each inner and outer hair cell bundle, and other cochlear structures visible in the images, was measured by bringing the structure into focus and determining the radial intersection angle from the horizontal axis. For each structure, the estimated motion for each phase of the stimulus cycle is plotted. These are fitted with a sinusoidal curve. The resulting fits for many cochlear structures are shown in Chapter 3.

2.6 Controls

After completing each run, the focal depth was raised to the plane of the apical bone, as shown in figure 2-9, and stroboscopic images taken at the same input stimulus level. This ensured that the entire cochlea was not moving and that the results obtained from the run were due to stapes displacement not shaking of the cochlea. Only experiments in which the apical bone motion measurements yielded no motion were considered valid.

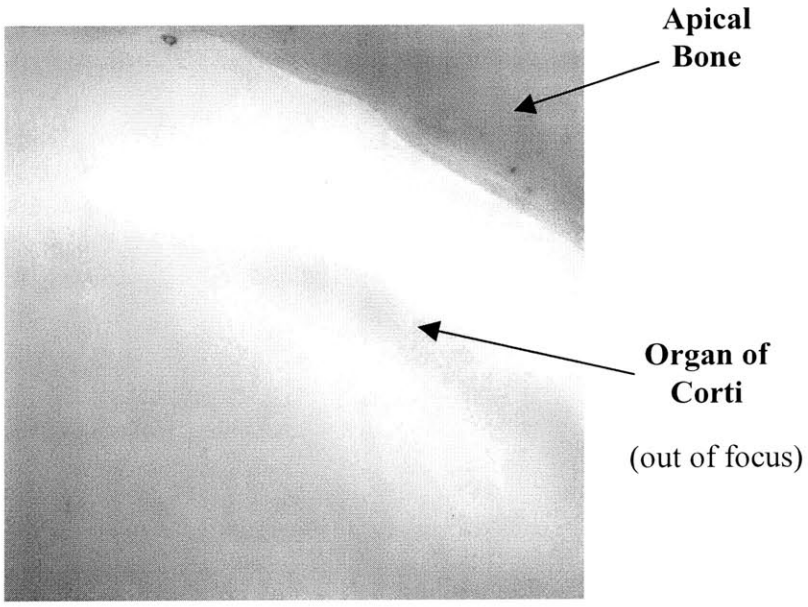


Figure 2-9: Apical bone at 40x magnification.

Chapter 3

Results

This chapter describes the image quality and motion measurements of various structures within the gerbil cochlea and the organ of Corti taken using the system described in chapter two.

3.1 Visible structures

Images taken in the apical portion of the organ of Corti reveal a variety of cochlear structures at various focal depths. Figure 3-1 shows a broad image of the Organ of Corti at 40x magnification using the procedure outlined in chapter two. The following figures 3-2 through 3-8 show the individual cochlear structures. These include inner hair cell bundles (Fig 3-2), pillar cells (Fig 3-3), outer hair cell bundles (Fig 3-4), the tectorial membrane (Fig 3-5), the intact Reissner's membrane (Fig 3-6), the tectorial membrane marginal band (Fig 3-7), and efferent fibers crossing the tunnel of Corti (Fig 3-8).

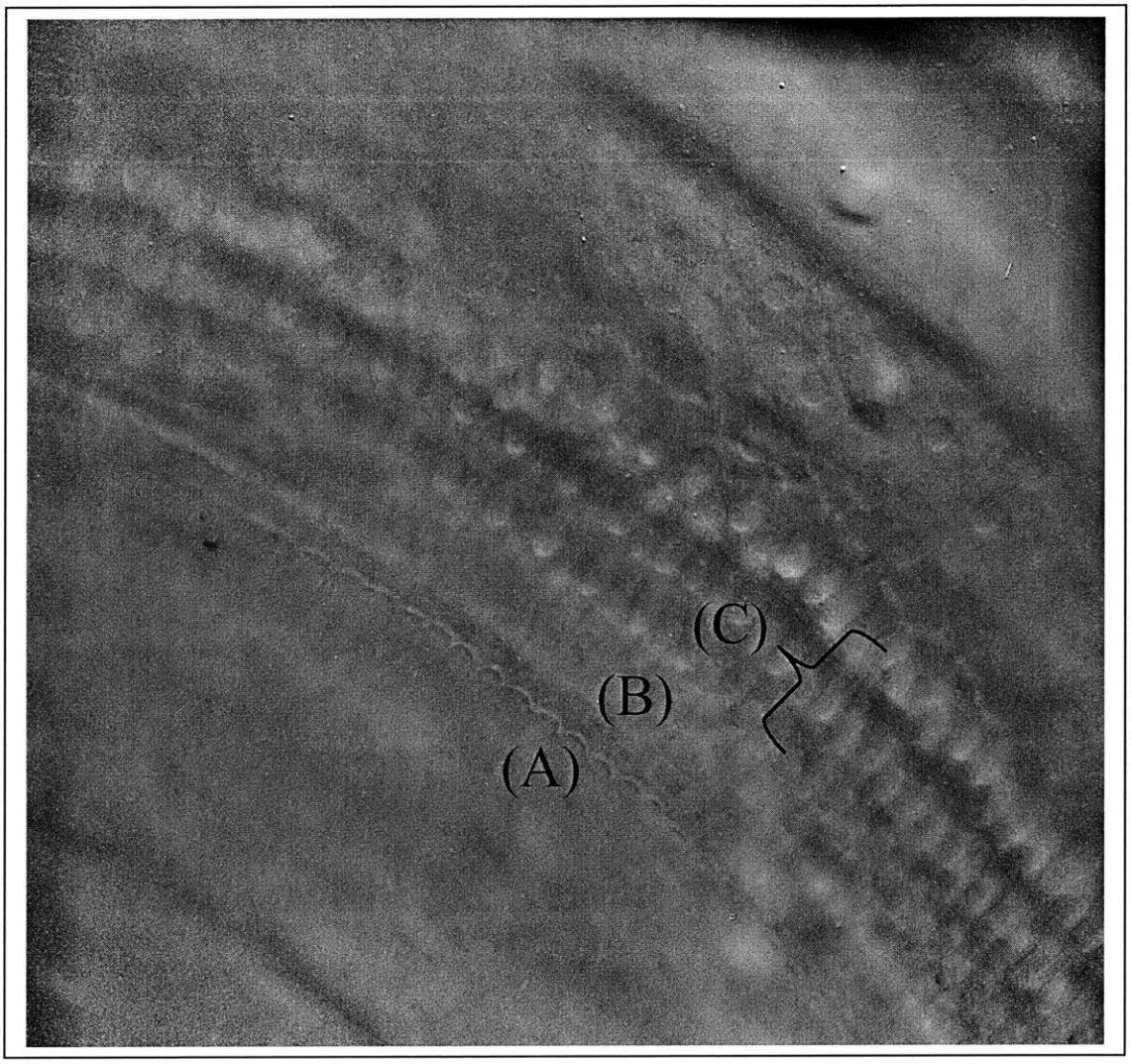
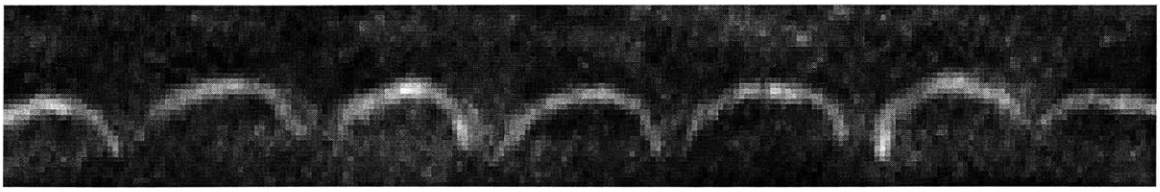


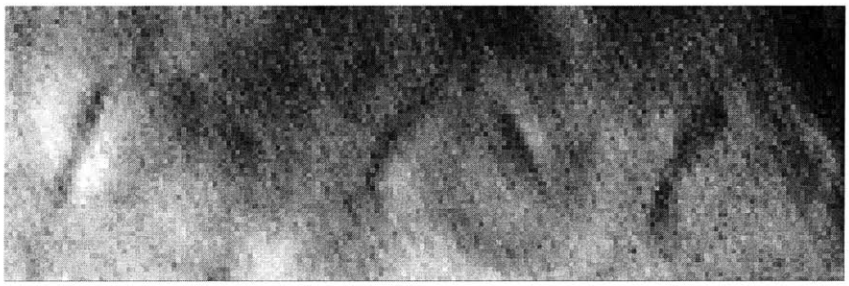
Figure 3-1: Apical turn of the organ of Corti at rest. The structure is viewed at 40X magnification through the apical hole, the intact Reissner's membrane, and the tectorial membrane. (A) Inner hair cell bundles. (B) Pillar cells. (C) Three rows of outer hair cells.

(A)

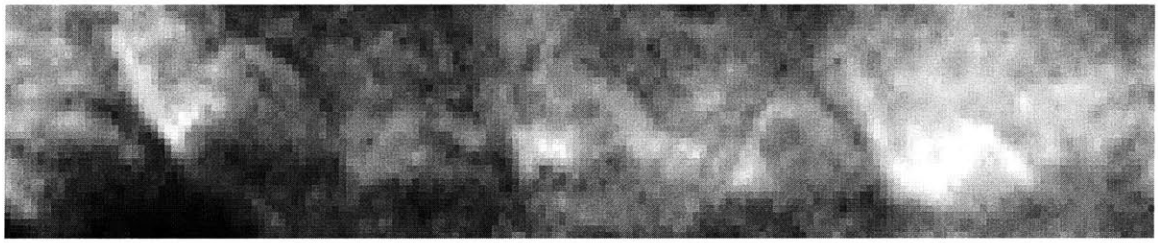


(B)

Figure 3-2: Inner hair cell bundles. (A) Inner hair cell bundles fixed in gluteraldahyde. (B) Unfixed inner hair cell bundles from the preparation.



(A)



(B)

Figure 3-3: Outer hair cell bundles. (A) Outer hair cell bundles fixed in gluteraldahyde. (B) Unfixed outer hair cell bundles from the preparation.

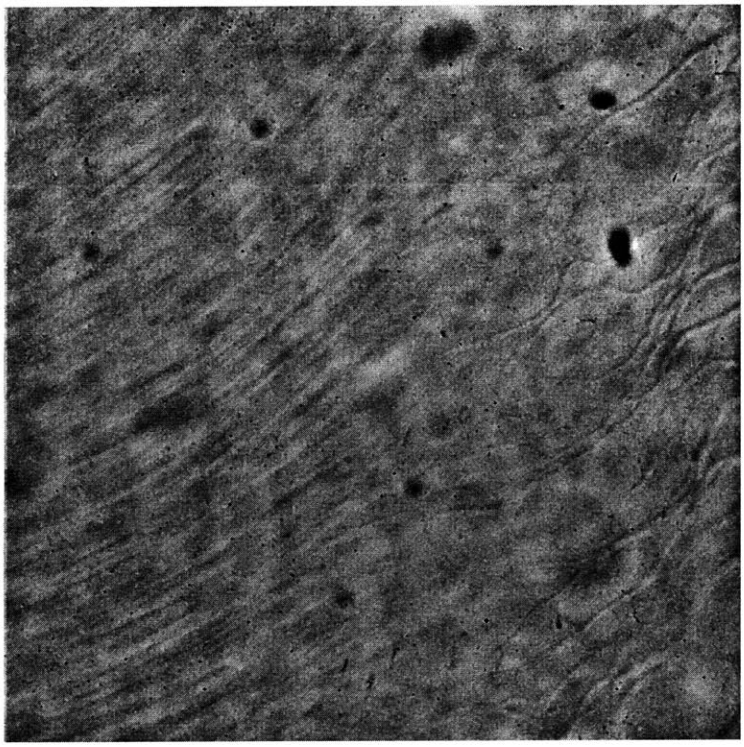


Figure 3-4: Tectorial Membrane. The Radial fibrillar structure can be seen on the left, while the covering net can be seen on the right.

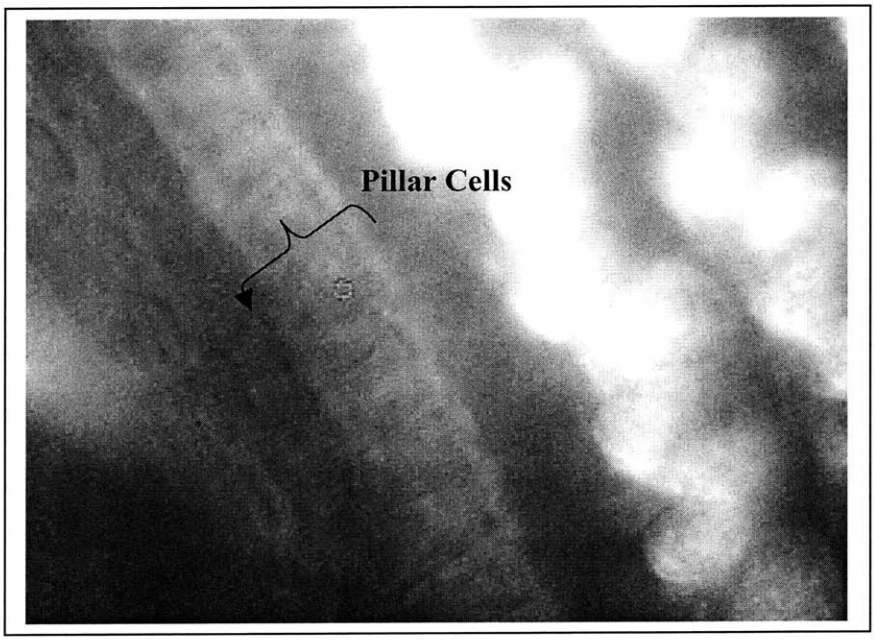


Figure 3-5: Pillar cells. The pillar cells sit between the outer hair cells on the right and the inner hair cells on the left.

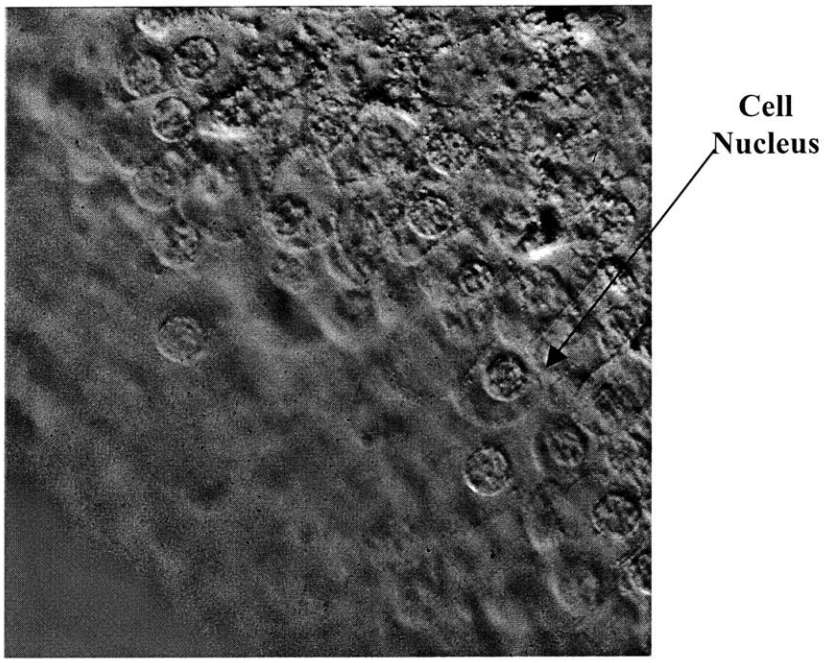


Figure 3-6: Reissner's membrane. As Reissner's membrane sits closest to the microscope objective, the high contrast allows cell nuclei to be easily seen.

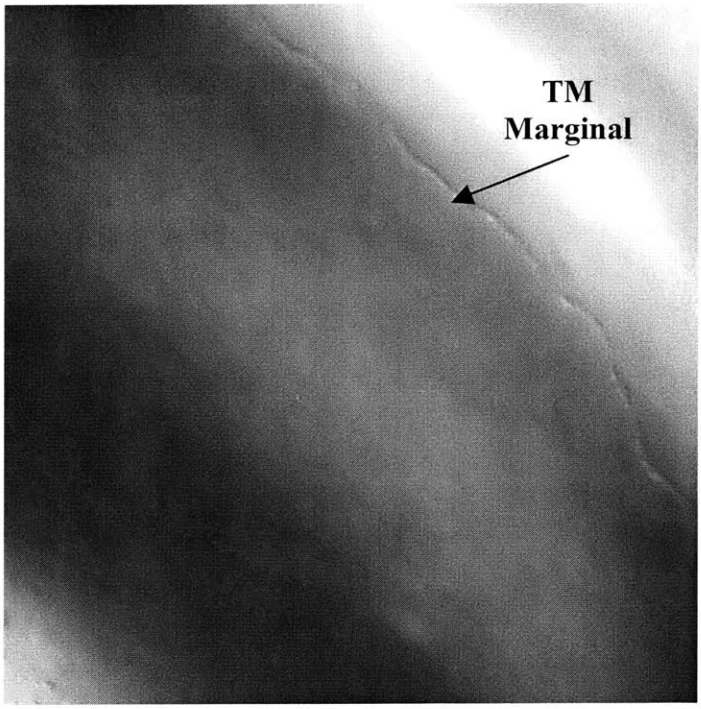


Figure 3-7: Marginal band of the tectorial membrane.

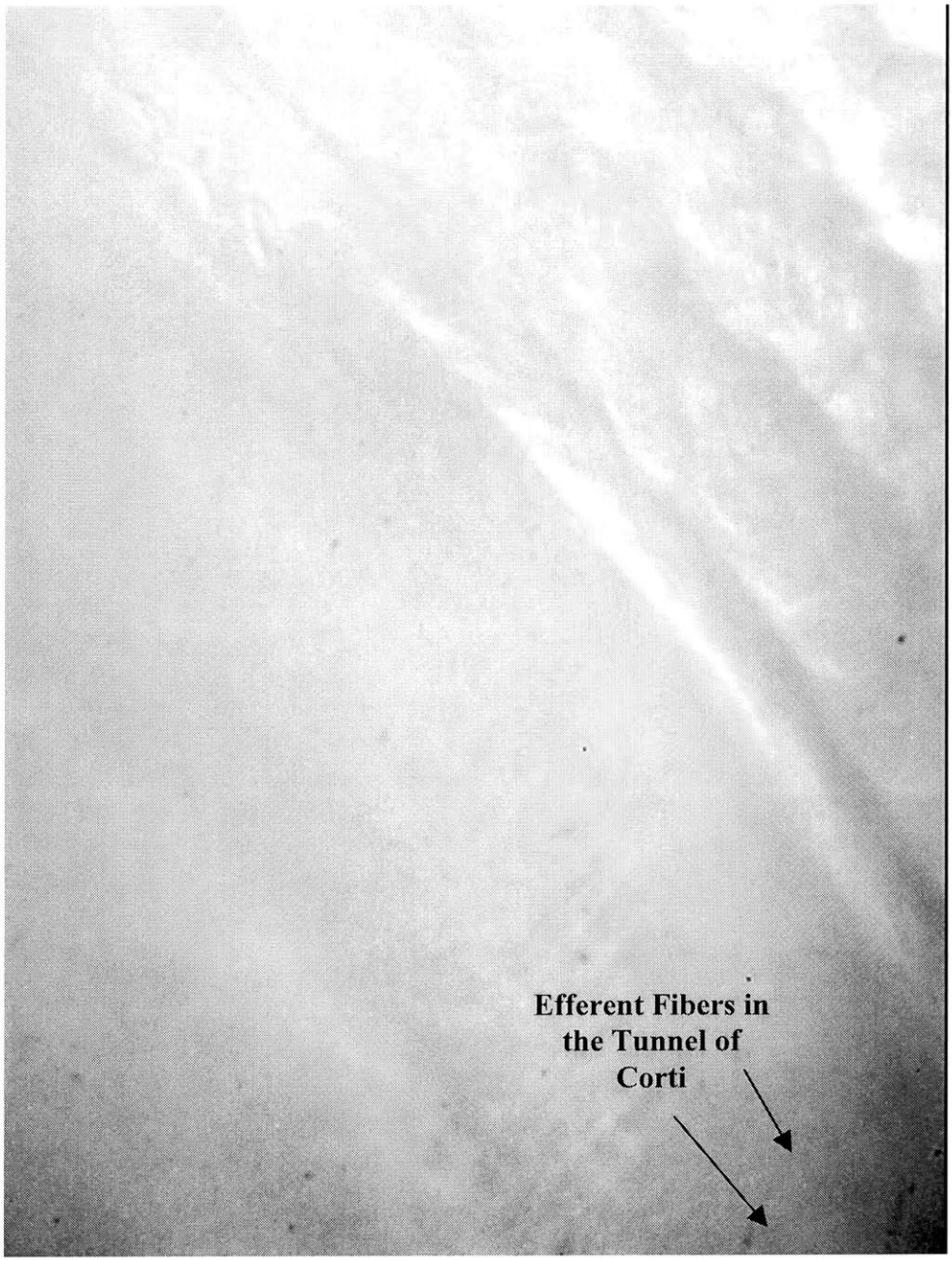
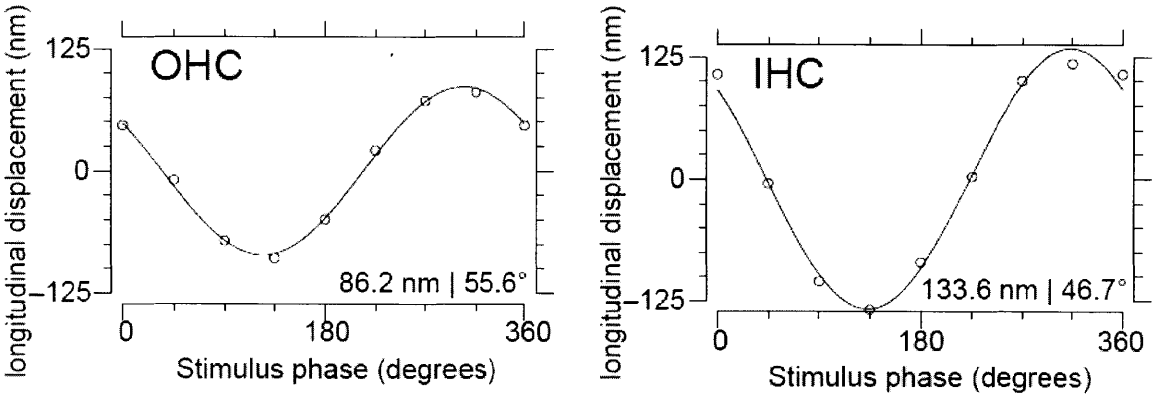


Figure 3-8: Efferent tunnel fibers crossing the tunnel of Corti.

3.2 Longitudinal Motion of the Organ of Corti

The organ of Corti is generally assumed to move primarily in the radial and transverse directions. However, longitudinal motion of the organ of Corti was observed across at least five preparations. Figure 3-9 shows the longitudinal motion of the organ of Corti for a representative inner and outer hair cell pair located on the same radial axis in one of the preparations. The motion measurement of the representative pair are fitted by sinusoidal curves, which shows that they are nearly in phase, with inner hair cell displacement about 50% larger than outer hair cell displacement.



(A) **(B)**

Figure 3-9: The longitudinal motion of one pair of representative inner and outer hair cell bodies located along the same radial axis. The stimulus was a 200 nm (~ 90 dB SPL) 400 Hz sinusoidal stapes displacement. (A) Outer hair cell shows a sinusoidal displacement with a magnitude of 86.2 nm and a phase of 55.6 degrees. (B) Inner hair cell shows a sinusoidal displacement with a magnitude of 133.6 nm and a phase of 46.7 degrees.

Typical motion measurements of the modiolus are shown in figure 3-10. These low magnitudes of approximately 7.1 nm in the radial direction and 4.6 nm in the longitudinal direction are believed to be stationary. This indicates that the longitudinal motion shown in figure 3-9 is specific to the organ of Corti.

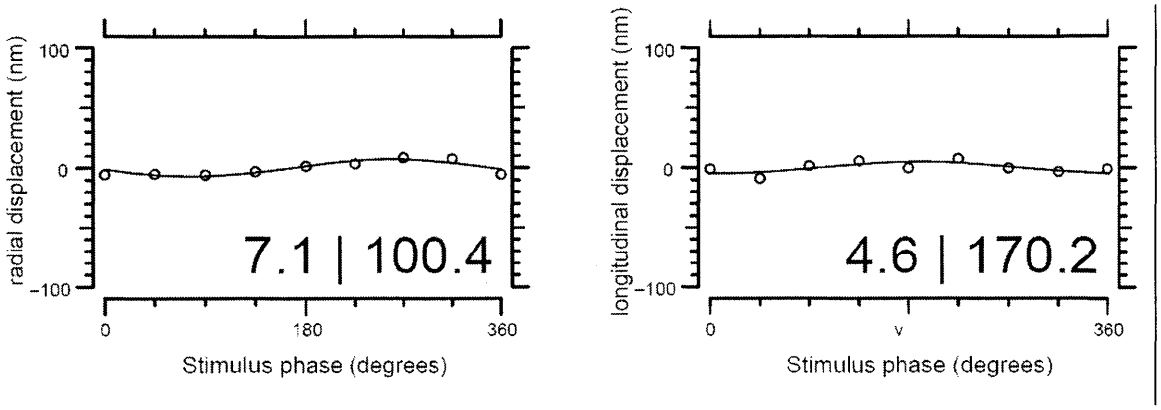


Figure 3-10: Typical motion of the modiolus. The modiolus measured a 7.1 nm displacement in the radial direction and 4.6 nm displacement in the longitudinal direction.

3.3 Radial Motion of Hair Cells and Bundles

Radial motion of the organ of Corti was observed in five preparations. Figure 3-11 shows the radial motion for representative inner and outer hair cell bundles and bodies located along the same radial axis in one preparation. The motion measurements of the representative pair are fitted by sinusoidal curves, which show similar magnitudes of motion and an approximate 15 degree phase lag of the outer hair cell body to the inner and outer hair cell bundles.

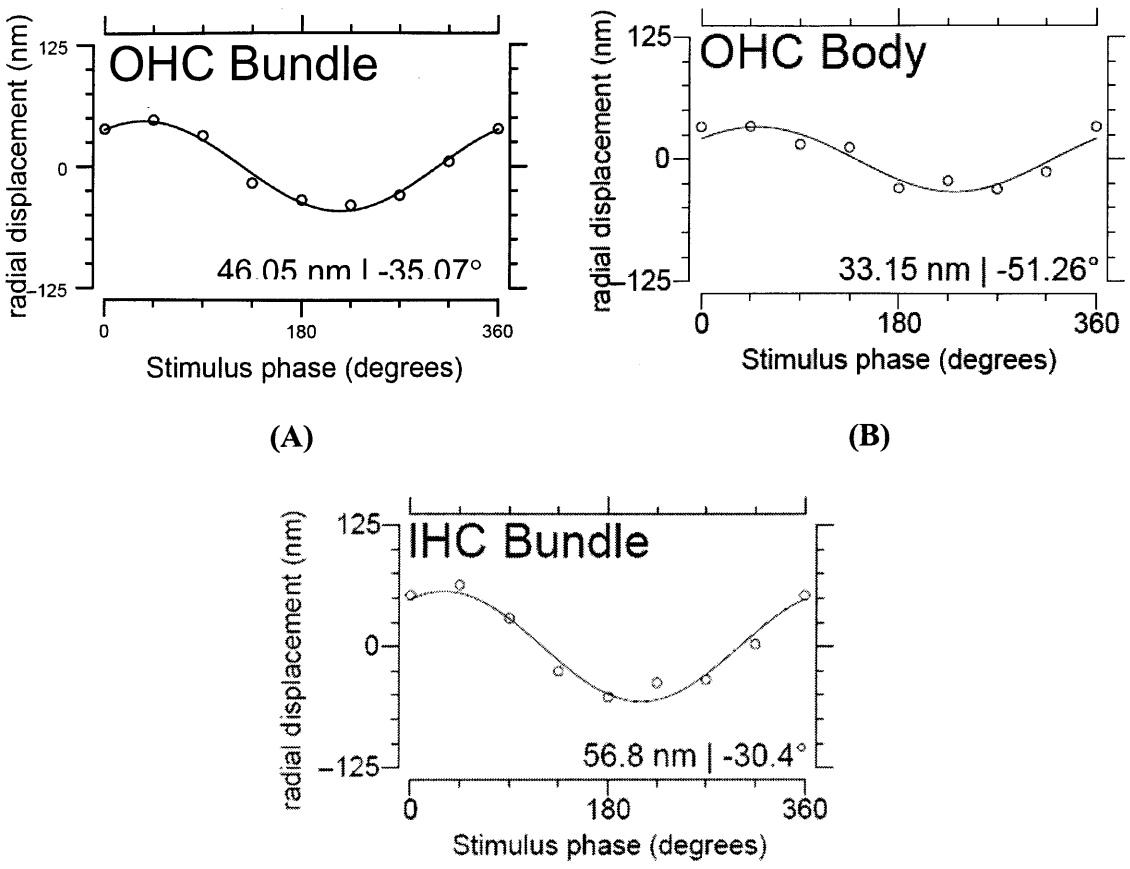


Figure 3-11: The radial motion of one pair of representative inner and outer hair cell bodies located along the same radial axis. The stimulus was a 200 nm (~ 90 dB SPL) 400 Hz sinusoidal stapes displacement. (A) Outer hair cell bundle shows a sinusoidal displacement with a magnitude of 46.05 nm and a phase of -35.07 degrees. (B) Outer hair cell body shows a sinusoidal displacement with a magnitude of 33.15 nm and a phase of -51.26 degrees. (C) Inner hair cell bundle shows a sinusoidal displacement with a magnitude of 56.8 nm and a phase of -30.4 degrees.

3.4 Motion of the Tectorial Membrane

Motion of the marginal band of the tectorial membrane was observed in two preparations. Figure 3-12 shows the longitudinal and radial motion of the marginal band. The motion measurements of the representative pair are fitted by sinusoidal curves, which show out of phase radial motion to that of the inner and outer hair cell bundles.

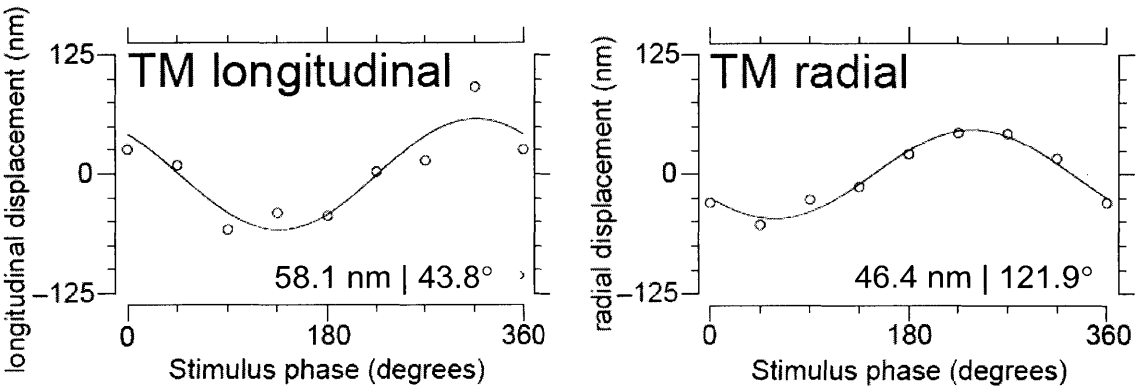


Figure 3-12: Motion of the tectorial membrane marginal band in response to ~200 nm 400 Hz sinusoidal stapes displacement, which corresponds to ~ 90dB SPL input sound stimuli. Radial motion is close to 180 degrees out of phase to that of inner and outer hair cell radial motion.

3.5 Longitudinal Motion of Efferent Tunnel Fibers

Figure 3-13 shows the longitudinal motion of efferent tunnel fibers crossing the tunnel of Corti compared to that of outer hair cell bodies at the same radial axis. Although the magnitudes of the motion are nearly the same, the tunnel fiber exhibits an approximate 30 degree phase lead to that of the outer hair cell bodies.

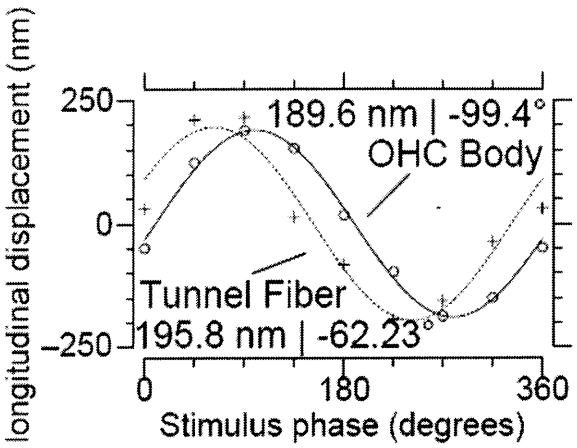


Figure 3-13: Motion of one pair of efferent fibers in the tunnel of Corti and outer hair cell bodies located along the same radial axis. The stimulus was a 420 nm (~97 dB SPL) 400 Hz sinusoidal stapes displacement. Radial motion is close to 180 degrees out of phase to that of inner and outer hair cell radial motion.

Chapter 4

Discussion

4.1 Motion Measurements

The measurements presented in chapter 3 provide some of the first direct measurements of the interaction between multiple structures in an intact cochlea. These include longitudinal motion of the organ of Corti, radial motion of the tectorial membrane relative to that of hair cell bundles, radial motions of hair cell bodies and bundles, and a longitudinal phase lead of efferent fibers in the tunnel of Corti relative to that of outer hair cell bodies. The results demonstrate that the cochlea is capable of more complex motions than have previously been shown.

The longitudinal motion, shown in figure 3-9, could provide an alternative method to that of transverse waves for energy propagation along the organ of Corti. The relative motion of the tectorial membrane with that of the hair cell bodies, shown in figures 3-11 and 3-12, may be indicative of mechanical resonance causing the tectorial membrane to shear against the hair bundles.

Figure 3-13 shows that the longitudinal motion of the efferent tunnel fibers leads that of the outer hair cell bodies and support a hypothesis (Hubbard et al, 2000) that fluid

flow in the tunnel of Corti is occurring. If this is the case, fluid flow due to a compression of the cochlear partition at a more basal location could cause fluid in the tunnel of Corti to propagate apically before the compression wave.

4.2 Stroboscopic light microscopy setup

There are certain advantages and disadvantages with any measurement system. While both the laser Doppler and confocal microscopy setups have better motion sensitivity in the transverse plane, stroboscopic light microscopy allows for a large number of structures to be imaged at once, thereby limiting the amount of time to obtain a measurement.

The time savings becomes increasingly important when the methods are applied in an *in vivo* preparation as cochlear sensitivity generally falls with time. Laser Doppler is already used with success with *in vivo* applications, but suffers from only being able to obtain one-dimensional motion measurements from an artificial reflector. Confocal microscopy is hard to realize *in vivo* due to physical constraints. Although physical constraints may require longer working distances *in vivo*, and thus lower resolutions, light microscopy may allow for multi dimensional motion measurements obtained with a relatively fast speed.

Chapter 5

Conclusion

We have developed a new technique to study cochlear micromechanics. The applied technique shows that the mechanical properties are more complex than previously believed. The combined measurement system and *in vitro* cochlear preparation enables high quality images of a variety of cochlear structures and motion measurements of those structures which may lead to improved understanding of the passive mechanics of the mammalian cochlea. One important caveat is that all measurements were taken with an uncovered apical viewing hole. Efforts are currently underway to seal the apical hole with glass to assess effects of the uncovered hole on longitudinal motion of the organ of Corti, out of phase motion between the outer hair cell bodies and their bundles, out of phase motion between the tectorial membrane marginal band and hair cell bundles, and a phase lead of the efferent fibers of the tunnel of Corti to the outer hair cell bodies.

Experience gained from this preparation may facilitate the transfer of this procedure to an *in vivo* preparation. If this is successful, the combined system and methods shown here would undoubtedly lead to exciting measurements in the field of cochlear micromechanics!

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Appendix

The following C-Shell script was developed to analyze the motion of a region of interest given by a ROI file. The script iteratively calls the optical flow algorithm *xyt* and sums the result of those calls. It then creates a set of stopped images based on the estimated motion it detected. It fits the motion to a sinusoidal curve and determines the magnitude and phase of that curve. The user can determine whether the motion measurement is reasonable by verifying that the stopped images are stopped, and verifying that the actual motion measurements for each phase of the stimulus cycle fit the estimated sinusoid.

```
#!/bin/csh -f

# parameters ------------------------------------------
set roifile = default
set pixelscalar = 1
set units = "pixels"
set prefix3D = "default"
set range = "0"
set postfix3D = ".3D"
set moveon = 1 # if set to 1 then creates stopped nD images
set phaserange = "000 001 002 003 004 005 006 007"
set postfixmoved = ".moved"
set angle = 0
set command = "newxyt"
set savespace = 1 # if set to 1 deletes intermediate files
set numavg = 5
set testnewnumbers = 0
set method = 1
set plane = 000
set movedname = "default"
set display = 0
set secondary = 0
set fold = 0
# -----------------------------------------------------

set goodrange = 0
set counter = 1
set nextcounter = 2

if ($#argv < 1) then
    echo "xytavg -i <inputprefix> -o <outputprefix> -r range(0.1 0.2) -
p plane -a angle -f roifile -s secondaryroi -n numavgs -x -y -xy"
```

```
    exit(0)
endif

while ($counter <= $#argv)
    switch  ($argv[$counter])
       case "-i":
          set prefix3D = $argv[$nextcounter]
          set counter = `echo "$counter + 2" | bc -l`
          set nextcounter = `echo "$nextcounter + 2" | bc -l`
          breaksw
       case "-o":
          set movedname = $argv[$nextcounter]
          set counter = `echo "$counter + 2" | bc -l`
          set nextcounter = `echo "$nextcounter + 2" | bc -l`
          breaksw
       case "-r":
          set range = $argv[$nextcounter]
          set counter = `echo "$counter + 2" | bc -l`
          set nextcounter = `echo "$nextcounter + 2" | bc -l`
          breaksw
       case "-h":
          echo "xytavg -i <inputprefix> -o <outputprefix> -r range(0.1
0.2) -p plane -a angle -f roifile -s secondaryroi -n numavgs -x -y -xy"
          exit(0)
          breaksw
       case "-p":
          set plane = $argv[$nextcounter]
          set counter = `echo "$counter + 2" | bc -l`
          set nextcounter = `echo "$nextcounter + 2" | bc -l`
          breaksw
       case "-a":
          set angle = $argv[$nextcounter]
          set counter = `echo "$counter + 2" | bc -l`
          set nextcounter = `echo "$nextcounter + 2" | bc -l`
          breaksw
        case "-f":
          set roifile = $argv[$nextcounter]
          set counter = `echo "$counter + 2" | bc -l`
          set nextcounter = `echo "$nextcounter + 2" | bc -l`
          breaksw
         case "-s":
          set sroifile = $argv[$nextcounter]
          set counter = `echo "$counter + 2" | bc -l`
          set secondary = 1
          set nextcounter = `echo "$nextcounter + 2" | bc -l`
          breaksw
        case "-t":
          set troifile = $argv[$nextcounter]
          set counter = `echo "$counter + 2" | bc -l`
          set nextcounter = `echo "$nextcounter + 2" | bc -l`
          set fold = 1
          breaksw
        case "-n":
          set numavg = $argv[$nextcounter]
          set counter = `echo "$counter + 2" | bc -l`
          set nextcounter = `echo "$nextcounter + 2" | bc -l`
          breaksw
```

```
       case "-16":
          set phaserange = "000 001 002 003 004 005 006 007 008 009 010
011 012 013 014 015"
          set counter = `echo "$counter + 1" | bc -l`
          set nextcounter = `echo "$nextcounter + 1" | bc -l`
          breaksw
         case "-x":
          set display = 1
          set counter = `echo "$counter + 1" | bc -l`
          set nextcounter = `echo "$nextcounter + 1" | bc -l`
          breaksw
         case "-y":
          set display = 2
          set counter = `echo "$counter + 1" | bc -l`
          set nextcounter = `echo "$nextcounter + 1" | bc -l`
          breaksw
         case "-xy":
          set display = 3
          set counter = `echo "$counter + 1" | bc -l`
          set nextcounter = `echo "$nextcounter + 1" | bc -l`
          breaksw
     endsw
end
echo "--------- XYTAVG INITIALIZE ---------------"
#rm -f $movedname*.y* ; rm -f $movedname*.x* ; rm -f $movedname*.ps ;
rm -f ?overlay.ps ; rm -f ymagnitude ; rm -f xmagnitude
rm -f $prefix3D*$postfixmoved*; rm -f xangle; rm -f yangle;
rm -f $movedname*

foreach mag ($range)
 set testnum = `echo "$mag * 1" | bc -l` # determines whether range is
a number for last magnitude display purposes
   if ($testnum =~ $mag) then
      set goodrange = 1
   endif
  break
end
echo "-------------------------------------------"

#set angle = 0

set originalpostfix3D = $postfix3D
foreach mag ($range)
   echo "Magnitude = $mag ..."
   set currentavg = 1
   set prevavg = 0
   set postfix3D = $originalpostfix3D

   3Dfrom2D $prefix3D$mag$postfix3D $prefix3D$mag.$plane.0??


   while ($currentavg <= $numavg)
    echo "Beginning pass $currentavg..."

    $command -y $prefix3D$mag$postfix3D $roifile | grep "y:" | grep -o
"\-\?.\......." > $movedname.$mag.y$currentavg
```

```
    $command -y $prefix3D$mag$postfix3D $roifile | grep "x:" | grep -o
"\-\?.\......." > $movedname.$mag.x$currentavg

    if ($currentavg =~ 1) then
       cp $movedname.$mag.y$currentavg $movedname.$mag.y
       cp $movedname.$mag.x$currentavg $movedname.$mag.x
    endif


    rm -f $movedname.$mag.ytemp.
    rm -f $movedname.$mag.xtemp
    set numphases = 0
    foreach phase ( $phaserange )

      set numphases = `echo "$numphases + 1" | bc`
      set line = `echo "$phase + 1" | bc -l`

      set ytemp = `sed -n {$line}p $movedname.$mag.y$currentavg`
      set yinv = `echo "$ytemp * -1" | bc -l`
      set xtemp = `sed -n {$line}p $movedname.$mag.x$currentavg`
      set xinv = `echo "$xtemp * -1" | bc -l`

      if ($currentavg > 1 ) then
         set oldy = `sed -n {$line}p $movedname.$mag.y`
         set oldx = `sed -n {$line}p $movedname.$mag.x`
         set sumy = `echo "$oldy + $ytemp" | bc -l`
         set invsumy = `echo "$sumy * -1" | bc -l`
         set sumx = `echo "$oldx + $xtemp" | bc -l`
         set invsumx = `echo "$sumx * -1" | bc -l`
         echo $sumy >> $movedname.$mag.ytemp
         echo $sumx >> $movedname.$mag.xtemp
      endif
      if ($moveon =~ 1) then
        if ($currentavg =~ 1) then
           if ($phase =~ 000) then
              cp $prefix3D$mag.$plane.$phase
$prefix3D$mag$postfixmoved$currentavg.$phase
           else
              movepic -- $prefix3D$mag.$plane.$phase
$prefix3D$mag$postfixmoved$currentavg.$phase $xinv $yinv 0
           endif
        else
           if ($phase =~ 000) then
              cp $prefix3D$mag.$plane.$phase
$prefix3D$mag$postfixmoved$currentavg.$phase
           else
              movepic -- $prefix3D$mag.$plane.$phase
$prefix3D$mag$postfixmoved$currentavg.$phase $invsumx $invsumy 0
           endif
           if ($savespace =~ 1) then
              rm -f $prefix3D$mag$postfixmoved$prevavg.$phase
              #rm -f $prefix3D$mag.y$prevavg
              #rm -f $prefix3D$mag.x$prevavg
              rm -f $prefix3D$mag$originalpostfix3D.$prevavg
           endif
           if ($currentavg =~ $numavg) then
```

```
            cp $prefix3D$mag$postfixmoved$currentavg.$phase
$movedname.$mag.$plane.$phase
          endif
        endif
      endif
    end
    if ($currentavg > 1) then
       rm -f $movedname.$mag.y
       rm -f $movedname.$mag.x
       mv $movedname.$mag.ytemp $movedname.$mag.y
       mv $movedname.$mag.xtemp $movedname.$mag.x
    endif

    set postfix3D = $originalpostfix3D.$currentavg

    3Dfrom2D $prefix3D$mag$postfix3D
$prefix3D$mag$postfixmoved$currentavg.0??

    set currentavg = `echo "$currentavg + 1" | bc -l`
    set prevavg = `echo "$prevavg + 1" | bc -l`
  end
  rm -f $movedname.s.*
  if ($secondary =~ 1) then  #have an additional roi to stop the motion
with
     echo "Secondary roi ..."
   set currentavg = 1
   set prevavg = 0
   set postfix3D = $originalpostfix3D

   3Dfrom2D $movedname.s.$mag$postfix3D $movedname.$mag.$plane.0??


   while ($currentavg <= $numavg)
    echo "Beginning pass $currentavg..."

    $command -y $movedname.s.$mag$postfix3D $sroifile | grep "y:" |
grep -o "\-\?.\......." > $movedname.s.$mag.y$currentavg
    $command -y $movedname.s.$mag$postfix3D $sroifile | grep "x:" |
grep -o "\-\?.\......." > $movedname.s.$mag.x$currentavg

    if ($currentavg =~ 1) then
       cp $movedname.s.$mag.y$currentavg $movedname.s.$mag.y
       cp $movedname.s.$mag.x$currentavg $movedname.s.$mag.x
    endif


    rm -f $movedname.s.$mag.ytemp.
    rm -f $movedname.s.$mag.xtemp
    set numphases = 0
    foreach phase ( $phaserange )

      set numphases = `echo "$numphases + 1" | bc`
      set line = `echo "$phase + 1" | bc -l`

      set ytemp = `sed -n {$line}p $movedname.s.$mag.y$currentavg`
      set yinv = `echo "$ytemp * -1" | bc -l`
      set xtemp = `sed -n {$line}p $movedname.s.$mag.x$currentavg`
```

```
      set xinv = `echo "$xtemp * -1" | bc -l`

      if ($currentavg > 1 ) then
         set oldy = `sed -n {$line}p $movedname.s.$mag.y`
         set oldx = `sed -n {$line}p $movedname.s.$mag.x`
         set sumy = `echo "$oldy + $ytemp" | bc -l`
         set invsumy = `echo "$sumy * -1" | bc -l`
         set sumx = `echo "$oldx + $xtemp" | bc -l`
         set invsumx = `echo "$sumx * -1" | bc -l`
         echo $sumy >> $movedname.s.$mag.ytemp
         echo $sumx >> $movedname.s.$mag.xtemp
      endif
      if ($moveon =~ 1) then
        if ($currentavg =~ 1) then
           if ($phase =~ 000) then
              cp $movedname.$mag.$plane.$phase
$movedname.s.$mag$postfixmoved$currentavg.$phase
           else
              movepic -- $movedname.$mag.$plane.$phase
$movedname.s.$mag$postfixmoved$currentavg.$phase $xinv $yinv 0
           endif
        else
           if ($phase =~ 000) then
              cp $movedname.$mag.$plane.$phase
$movedname.s.$mag$postfixmoved$currentavg.$phase
           else
              movepic -- $movedname.$mag.$plane.$phase
$movedname.s.$mag$postfixmoved$currentavg.$phase $invsumx $invsumy 0
           endif
           if ($savespace =~ 1) then
              rm -f $movedname.s.$mag$postfixmoved$prevavg.$phase
              #rm -f $movedname.s.$mag.y$prevavg
              #rm -f $movedname.s.$mag.x$prevavg
              rm -f $movedname.s.$mag$originalpostfix3D.$prevavg
           endif
           if ($currentavg =~ $numavg) then
             cp $movedname.s.$mag$postfixmoved$currentavg.$phase
$movedname.s.$mag.$plane.$phase
           endif
        endif
      endif
    end
    if ($currentavg > 1) then
       rm -f $movedname.s.$mag.y
       rm -f $movedname.s.$mag.x
       mv $movedname.s.$mag.ytemp $movedname.s.$mag.y
       mv $movedname.s.$mag.xtemp $movedname.s.$mag.x
    endif

    set postfix3D = $originalpostfix3D.$currentavg

    3Dfrom2D $movedname.s.$mag$postfix3D
$movedname.s.$mag$postfixmoved$currentavg.0??

    set currentavg = `echo "$currentavg + 1" | bc -l`
    set prevavg = `echo "$prevavg + 1" | bc -l`
   end
```

```
endif

if ($fold =~ 1) then  #fold back on first one
    echo "Tertiary roi ..."
  set currentavg = 1
  set prevavg = 0
  set postfix3D = $originalpostfix3D

  3Dfrom2D $movedname.t.$mag$postfix3D $movedname.s.$mag.$plane.0??


  while ($currentavg <= $numavg)
   echo "Beginning pass $currentavg..."

   $command -y $movedname.t.$mag$postfix3D $troifile | grep "y:" |
grep -o "\-\?.\......." > $movedname.t.$mag.y$currentavg
   $command -y $movedname.t.$mag$postfix3D $troifile | grep "x:" |
grep -o "\-\?.\......." > $movedname.t.$mag.x$currentavg

   if ($currentavg =~ 1) then
      cp $movedname.t.$mag.y$currentavg $movedname.t.$mag.y
      cp $movedname.t.$mag.x$currentavg $movedname.t.$mag.x
   endif


   rm -f $movedname.t.$mag.ytemp.
   rm -f $movedname.t.$mag.xtemp
   set numphases = 0
   foreach phase ( $phaserange )

     set numphases = `echo "$numphases + 1" | bc`
     set line = `echo "$phase + 1" | bc -l`

     set ytemp = `sed -n {$line}p $movedname.t.$mag.y$currentavg`
     set yinv = `echo "$ytemp * -1" | bc -l`
     set xtemp = `sed -n {$line}p $movedname.t.$mag.x$currentavg`
     set xinv = `echo "$xtemp * -1" | bc -l`

     if ($currentavg > 1 ) then
        set oldy = `sed -n {$line}p $movedname.t.$mag.y`
        set oldx = `sed -n {$line}p $movedname.t.$mag.x`
        set sumy = `echo "$oldy + $ytemp" | bc -l`
        set invsumy = `echo "$sumy * -1" | bc -l`
        set sumx = `echo "$oldx + $xtemp" | bc -l`
        set invsumx = `echo "$sumx * -1" | bc -l`
        echo $sumy >> $movedname.t.$mag.ytemp
        echo $sumx >> $movedname.t.$mag.xtemp
     endif
     if ($moveon =~ 1) then
       if ($currentavg =~ 1) then
          if ($phase =~ 000) then
             cp $movedname.s.$mag.$plane.$phase
$movedname.t.$mag$postfixmoved$currentavg.$phase
          else
             movepic -- $movedname.s.$mag.$plane.$phase
$movedname.t.$mag$postfixmoved$currentavg.$phase $xinv $yinv 0
          endif
```

```
        else
           if ($phase =~ 000) then
              cp $movedname.s.$mag.$plane.$phase
$movedname.t.$mag$postfixmoved$currentavg.$phase
           else
              movepic -- $movedname.s.$mag.$plane.$phase
$movedname.t.$mag$postfixmoved$currentavg.$phase $invsumx $invsumy 0
           endif
           if ($savespace =~ 1) then
              rm -f $movedname.t.$mag$postfixmoved$prevavg.$phase
              #rm -f $movedname.t.$mag.y$prevavg
              #rm -f $movedname.t.$mag.x$prevavg
              rm -f $movedname.t.$mag$originalpostfix3D.$prevavg
           endif
           if ($currentavg =~ $numavg) then
             cp $movedname.t.$mag$postfixmoved$currentavg.$phase
$movedname.t.$mag.$plane.$phase
           endif
        endif
      endif
    end
    if ($currentavg > 1) then
       rm -f $movedname.t.$mag.y
       rm -f $movedname.t.$mag.x
       mv $movedname.t.$mag.ytemp $movedname.t.$mag.y
       mv $movedname.t.$mag.xtemp $movedname.t.$mag.x
    endif

    set postfix3D = $originalpostfix3D.$currentavg

    3Dfrom2D $movedname.t.$mag$postfix3D
$movedname.t.$mag$postfixmoved$currentavg.0??

    set currentavg = `echo "$currentavg + 1" | bc -l`
    set prevavg = `echo "$prevavg + 1" | bc -l`
   end
 endif




   foreach phase ( $phaserange )
      set line = `echo "$phase + 1" | bc -l`
      if ($secondary =~ 1) then
         set yraw1 = `sed -n {$line}p $movedname.$mag.y`
         set xraw1 = `sed -n {$line}p $movedname.$mag.x`
         set yraw2 = `sed -n {$line}p $movedname.s.$mag.y`
         set xraw2 = `sed -n {$line}p $movedname.s.$mag.x`
         if ($fold =~ 1) then
            set yraw3 = `sed -n {$line}p $movedname.t.$mag.y`
            set xraw3 = `sed -n {$line}p $movedname.t.$mag.x`
            set yraw = `echo "$yraw1 + $yraw2 + $yraw3" | bc -l`
            set xraw = `echo "$xraw1 + $xraw2 + $xraw3" | bc -l`
         else
            set yraw = `echo "$yraw1 + $yraw2" | bc -l`
            set xraw = `echo "$xraw1 + $xraw2" | bc -l`
```

```
         endif
      else
         set yraw = `sed -n {$line}p $movedname.$mag.y`
         set xraw = `sed -n {$line}p $movedname.$mag.x`
      endif
       set yinv = `echo "$yraw * -1" | bc -l`
       set xinv = `echo "$xraw * -1" | bc -l`
       if ($phase =~ 000) then
              cp $prefix3D$mag.$plane.$phase
$movedname.final.$mag.$plane.$phase
       else
              movepic -- $prefix3D$mag.$plane.$phase
$movedname.final.$mag.$plane.$phase $xinv $yinv 0
       endif


     set xtemp = `echo "(($xraw * c($angle / 360 * 2 * 4 * a(1))) -
($yraw * s($angle / 360 * 2 * 4 * a(1)))) * $pixelscalar" | bc -l`
     set ytemp = `echo "(($xraw * s($angle / 360 * 2 * 4 * a(1))) +
($yraw * c($angle / 360 * 2 * 4 * a(1)))) * $pixelscalar" | bc -l`
      echo $ytemp >> $movedname.$mag.yang
      echo $xtemp >> $movedname.$mag.xang
   end
   echo 0 >> $movedname.$mag.yang
   echo 0 >> $movedname.$mag.xang

   datatops -yy $movedname.$mag.yang
   datatops -yy $movedname.$mag.xang

   set yavg = `head -n $numphases $movedname.$mag.yang | awk '{sum+=$1}
END {print sum/NR}'`
   set xavg = `head -n $numphases $movedname.$mag.xang | awk '{sum+=$1}
END {print sum/NR}'`
   cat $movedname.$mag.yang | awk -v yavg=$yavg '{print $1-yavg}' >
$movedname.$mag.y.nodc.temp
   cat $movedname.$mag.xang | awk -v xavg=$xavg '{print $1-xavg}' >
$movedname.$mag.x.nodc.temp

   echo > $movedname.$mag.y.nodc ; echo > $movedname.$mag.x.nodc ; rm -
f $movedname.$mag.x.nodc ; rm -f $movedname.$mag.y.nodc
   foreach phase ( $phaserange )
      set line = `echo "$phase + 1" | bc -l`
      set ytemp = `sed -n {$line}p $movedname.$mag.y.nodc.temp`
      set xtemp = `sed -n {$line}p $movedname.$mag.x.nodc.temp`
      echo "`echo '$phase * 360 / $numphases' | bc -l` $xtemp" >>
$movedname.$mag.x.nodc
      echo "`echo '$phase * 360 / $numphases' | bc -l` $ytemp" >>
$movedname.$mag.y.nodc
   end
   echo "360 `sed -n 1p $movedname.$mag.y.nodc.temp`" >>
$movedname.$mag.y.nodc
   echo "360 `sed -n 1p $movedname.$mag.x.nodc.temp`" >>
$movedname.$mag.x.nodc


   rm -f $movedname.$mag.y.nodc.temp
   rm -f $movedname.$mag.x.nodc.temp
```

```
   datatops $movedname.$mag.y.nodc -y linear $units
   datatops $movedname.$mag.x.nodc -y linear $units


   set ymag =   `head -n $numphases $movedname.$mag.y.nodc | awk
'{print $2}' | 1dfft | awk '{print $2}' | head -n 1`
   set yangle =   `head -n $numphases $movedname.$mag.y.nodc | awk
'{print $2}' | 1dfft | awk '{print $3}' | head -n 1`
   set xmag =   `head -n $numphases $movedname.$mag.x.nodc | awk
'{print $2}' | 1dfft | awk '{print $2}' | head -n 1`
   set xangle =   `head -n $numphases $movedname.$mag.x.nodc | awk
'{print $2}' | 1dfft | awk '{print $3}' | head -n 1`

   echo "/mag $ymag def" > $movedname.$mag.y.ps
   echo "/phase $yangle def" >> $movedname.$mag.y.ps
   echo "/mag $xmag def" > $movedname.$mag.x.ps
   echo "/phase $xangle def" >> $movedname.$mag.x.ps
   head -n `cat $movedname.$mag.y.nodc.ps | awk ' END {print NR - 1}'`
$movedname.$mag.y.nodc.ps >> $movedname.$mag.y.ps
   head -n `cat $movedname.$mag.x.nodc.ps | awk ' END {print NR - 1}'`
$movedname.$mag.x.nodc.ps >> $movedname.$mag.x.ps
   echo "1 linetype beginplot" >> $movedname.$mag.y.ps
   echo "0 1 360 {dup phase add cos mag mul plot} for" >>
$movedname.$mag.y.ps
   echo "endplot" >> $movedname.$mag.y.ps
   echo "showpage" >> $movedname.$mag.y.ps
   echo "1 linetype beginplot" >> $movedname.$mag.x.ps
   echo "0 1 360 {dup phase add cos mag mul plot} for" >>
$movedname.$mag.x.ps
   echo "endplot" >> $movedname.$mag.x.ps
   echo "showpage" >> $movedname.$mag.x.ps

   echo "$ymag $yangle" > $movedname.$mag.y.info
   echo "$xmag $xangle" > $movedname.$mag.x.info


    switch  ($display)
       case 1:
          gv $movedname.$mag.x.ps &
          breaksw
       case 2:
          gv $movedname.$mag.y.ps &
          breaksw
       case 3:
          gv $movedname.$mag.x.ps &
          gv $movedname.$mag.y.ps &
          breaksw

    endsw


    set df = `echo "2 * 4 * a(1) / $numphases" | bc -l`
    set realy = 0
    set realx = 0
    set imagy = 0
    set imagx = 0
```

```
    set f = 0
    foreach phase ( $phaserange )
      set line = `echo "$phase + 1" | bc -l`
      set yraw = `sed -n {$line}p $movedname.$mag.y`
      set xraw = `sed -n {$line}p $movedname.$mag.x`
      set xtemp = `echo "($xraw * c($angle / 360 * 2 * 4 * a(1))) -
($yraw * s($angle / 360 * 2 * 4 * a(1)))" | bc -l`
      set ytemp = `echo "($xraw * s($angle / 360 * 2 * 4 * a(1))) +
($yraw * c($angle / 360 * 2 * 4 * a(1)))" | bc -l`
      set realy = `echo "$realy + ( $ytemp * c( $f ))" | bc -l`
      set realx = `echo "$realx + ( $xtemp * c( $f ))" | bc -l`
      set imagy = `echo "$imagy - ( $ytemp * s( $f ))" | bc -l`
      set imagx = `echo "$imagx - ( $xtemp * s( $f ))" | bc -l`
      set f = `echo "$f + $df"`
    end
    set realx = `echo "( $realx + $realx ) / $numphases" | bc -l`
    set realy = `echo "( $realy + $realy ) / $numphases" | bc -l`
    set imagx = `echo "( $imagx + $imagx ) / $numphases" | bc -l`
    set imagy = `echo "( $imagy + $imagy ) / $numphases" | bc -l`
    set ymag = `echo "sqrt(( $realy * $realy ) + ( $imagy * $imagy ))"
| bc -l`
    set yangle = `echo "360 / (2 * 4 * a(1)) * a( $imagy/$realy )" | bc
-l`
    set xmag = `echo "sqrt(( $realx * $realx ) + ( $imagx * $imagx ))"
| bc -l`
    set xangle = `echo "360 / (2 * 4 * a(1)) * a( $imagx/$realx )" | bc
-l`
    echo $xmag > xtemp
    echo $ymag > ytemp
    echo $xangle >> xangle
    echo $yangle >> yangle
    #set longcomp = `echo "($xtemp * c($angle / 360 * 2 * 4 * a(1))) -
($ytemp * s($angle / 360 * 2 * 4 * a(1)))" | bc -l`
    #set radialcomp = `echo "($xtemp * s($angle / 360 * 2 * 4 * a(1)))
+ ($ytemp * c($angle / 360 * 2 * 4 * a(1)))" | bc -l`

    #echo $longcomp >> $prefix3D$mag.lon
    #echo $radialcomp >> $prefix3D$mag.rad




    #$command -s $angle $prefix3D$mag$postfix3D $roifile | grep -A 1 y:
| grep harmonic | grep -o "\-\?.\....... pixels" | grep -o "\-
\?.\......." > ytemp
    #$command -s $angle $prefix3D$mag$postfix3D $roifile | grep -A 1 x:
| grep harmonic | grep -o "\-\?.\....... pixels" | grep -o "\-
\?.\......." > xtemp

    echo $pixelscalar >> ytemp ; echo $pixelscalar >> xtemp ; echo \*
>> ytemp ; echo \* >> xtemp ; echo p >> ytemp ; echo p >> xtemp
   if ($goodrange =~ 1) then
       echo $mag >> xmagnitude; echo $mag >> ymagnitude
   endif
    dc ytemp >> ymagnitude ; dc xtemp >> xmagnitude
```

```
    echo "xmagnitude: $xmag xangle: $xangle ymagnitude: $ymag yangle:
$yangle"


end

if ($goodrange =~ 1) then
   datatops -xyxy ymagnitude -y linear $units
   datatops -xyxy xmagnitude -y linear $units
else
   datatops -yy ymagnitude -y linear $units
   datatops -yy xmagnitude -y linear $units
endif

# Overlay Plot Stuff
#overlayplots new.0.9.y.ps new.0.[1-8].y.ps new.1.ps > yoverlay.ps
#overlayplots new.0.9.x.ps new.0.[1-8].x.ps new.1.ps > xoverlay.ps
# ------------------------

switch ($display)
   case 1:
      #gv xoverlay.ps &
     # gv xmagnitude.ps &
      breaksw
   case 2:
      # gv yoverlay.ps &
      # gv ymagnitude.ps &
       breaksw
   case 3:
      # gv xoverlay.ps &
       #gv xmagnitude.ps &
      # gv yoverlay.ps &
       #gv ymagnitude.ps &
endsw
```