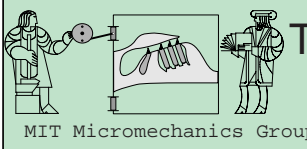


The micromechanical properties of the inner ear are poorly understood in any species. We are investigating these properties in the isolated basilar papilla of the alligator lizard. We use a combination of video microscopy, stroboscopic illumination, and computer vision to determine sound-induced displacements of papillar structures with a resolution of 1 nanometer. From images taken at multiple planes of focus we can tomographically reconstruct the three-dimensional shape of the papilla at each measured phase of sound stimulation. Tomographic reconstruction is made possible by increasing the numerical aperture of the optical system to more than double axial resolution. With new surgical techniques the preparation is stable for more than 12 hours, providing time to acquire three-dimensional images at multiple stimulus frequencies. We can thus compute arbitrary cross-sectional views through the organ as it moves in response to sound at multiple frequencies, while keeping the papilla physically intact. The ability to measure three-dimensional motions of many structures simultaneously has led to new insights about the interplay of structures in cochlear micromechanics. Previous studies¹⁻⁴ provide evidence that the motions of hair bundles and of the basilar papilla depend on both frequency and location. However, these studies measured motion in only one dimension for a limited number of locations on the endolymphatic surface. We corroborate and extend their results by showing that (1) papillar motions can be either elliptical or pistonlike; (2) motions in each direction vary with frequency and position; (3) these variations with frequency and position can differ for each direction of motion. Previous models^{5,7} have suggested that the basilar papilla undergoes a simple rocking mode of motion in which lateral displacement of the papilla drives hair bundle deflection. Our results suggest that additional modes of motion could contribute to the frequency selectivity of hair cells in this organ.



Massachusetts Institute of Technology Massachusetts Eye and Ear Infirmary Harvard/MIT Speech and Hearing Sciences Program

Tomographic Reconstruction of Three-Dimensional Cochlear Motions

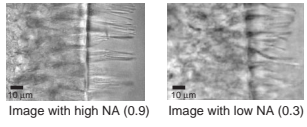
A.J. Aranyosi and Dennis M. Freeman



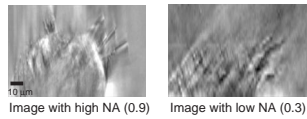
WHY 3-D?
Cochlear structures move in 3-D
1-D and 2-D measurements can be ambiguous

HIGHER NA = SHARPER IMAGES

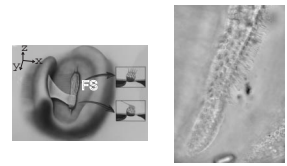
Brightfield microscopy resolution depends on NA



Axial resolution critically depends on NA



ALLIGATOR LIZARD COCHLEA



Planar — easier to image
Measured motions in the free-standing region (FS) can be compared to models (Freeman and Weiss, 1988, 1990)

3-D RECONSTRUCTION

1 ACQUIRE IMAGES

x-y-plane images
× multiple z planes
3-D images

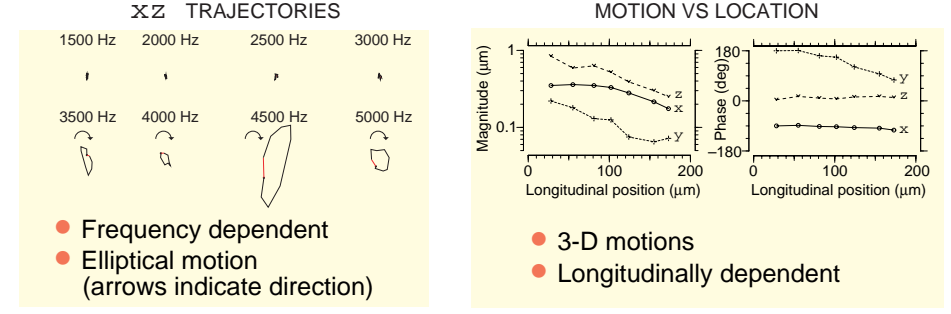
2 RESLICE 3-D IMAGES

x-z plane shows hair bundle rotation
y-z plane shows tonotopic organization

3 MEASURE MOTION

take 3-D images at several phases of sound stimulus
measure motions of hair bundles and reticular lamina (at the base of hair bundles) from images

THE RETICULAR LAMINA IS MECHANICALLY TUNED



HAIR BUNDLE ROTATION IS FREQUENCY DEPENDENT

MEASURING ROTATION

HAIR BUNDLE TUNING CURVE

WHAT DO HAIR BUNDLES SENSE?

Hair bundle rotation is determined by subtracting RL motion from tip motion

At this frequency (1.3 kHz) and for these bundles

- Tips of bundles move more than the RL
- As the RL moves right, the bundle rotates left
- The hair bundles rotate by about 1°
- At high pressures, hair bundles are broadly tuned mechanically
- Tuning is similar to ANFs at high pressures
- Hair bundle rotations are frequency dependent
- Rotation magnitudes match predictions of Freeman and Weiss
- Phases don't match

DISCUSSION

- SIGNIFICANCE FOR STUDYING COCHLEAR MECHANICS
- high-resolution images of living tissue
 - can measure sound-induced motions in 3-D
 - virtual cross-sections let us view motion from multiple angles
 - motions of all visible structures can be measured simultaneously
 - can experimentally test models of cochlear mechanics

- SIGNIFICANCE FOR LIZARD HEARING
- Preliminary mechanical measurements suggest
- RL contributes to frequency selectivity
 - RL contributes to tonotopic organization
 - Mechanical tuning accounts for neural tuning at high sound pressures
 - Fluid coupling dominates hair bundle response to RL motion

Supported by NIH Research Grant R01-DC00238. D.M. Freeman was supported in part by the W.M. Keck Career Development Professorship

References

1. Aranyosi, Davis, and Freeman, ARO 1998
2. Freeman and Weiss, *Hearing Research* 35, 1988
3. Freeman and Weiss, *Hearing Research* 48, 1990
4. Frischopt and DeRosier, *Hearing Research* 12, 1983
5. Holton and Hudspeth, *Science* 222, 1983
6. Holton and Weiss, *J. Physiol* 345, 1983
7. Weiss and Leono, *Hearing Research* 20, 1985