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The Digital Cytocochleogram

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Abstract

The Mouse Cochlea Database (MCD) is a collection of resources that include digital images and bibliographic information on the mouse cochlea and is available at: http://mousecochlea.ccgb.umn.edu. The purpose of this communication is to report on the development of one MCD resource: the Digital Cytocochleogram. A cytocochleogram is a graphic representation of the anatomical state of the hair cells along the complete width and length of the organ of Corti. The Digital Cytocochleogram provides Internet users with a complete collection of digital images of one or more surface preparations of the mouse organ of Corti from which morphometric information can be obtained. By moving a mouse driven, screen cursor over a digital image, the location and approximate frequency region of the anatomical structure is displayed. Users can also measure the straight-line distance between any two structures on the image. The Digital Cytocochleogram resource uses two software programs, the Coordinate Finder and Viewer, which are written as CGI scripts. The Coordinate Finder program maps each digital image to an X,Y coordinate system. The total length of the organ of Corti from all tissue segments is computed using an arc-distance approximation formula, with the lateral border of the inner pillar cell headplates serving as a trace line or reference location. After all of the digital images of the tissue segments are mapped, they are placed on the MCD Website where users can use the Viewer program to view and morphometrically assess structures using a web browser. A single, complete surface preparation from a normal mouse is presently available on the MCD website. As the MCD grows, additional images of surface preparations at different magnifications from normal, mutant, and experimentally altered mouse cochleas will become available.

1. Introduction

In 1966, Engstrom and coworkers described a method for producing surface preparations of the organ of Corti and a 2-dimensional graphic representation of damaged hair cells along the length of the basilar membrane. This graphic record was called a cochleogram or cytocochleogram. Its purpose was to relate hair-cell damage with frequency specific changes in hearing thresholds following acoustic trauma or ototoxic drug exposure.

Subsequently, a variety of methods have been used to produce surface preparations of the organ of Corti and to represent structural damage in the form of a cytocochleogram. Whole-mount, surface preparations of the organ of Corti/basilar membrane complex are usually prepared from chemically fixed tissues. Decalcified, dissected tissues can be mounted on a slide in aqueous media (Engstrom et al., 1966; Santi and Muchow, 1979), from undecalcified, plastic-embedded tissues (Bohne, 1972; Santi, 1986), or from Scanning Electron Microscopic preparations (Hunter-Duvar, 1978). However, plastic-embedded tissues offer the best advantages of tissue morphology and preservation of structural details. An important factor for producing surface preparations is that all of the organ of Corti with the corresponding hair cells must be harvested in order to relate structural damage with the frequency/distance map of the basilar membrane. Engstrom et al. (1966) represented the four rows of hair cells as open (i.e., normal cells) and filled (i.e., damaged cells) circles. Other investigators have

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Abbreviations: CGI, common gateway interface; MCD, mouse cochlea database; NIDCD, national institute on deafness and other communication disorders; PHP, hypertext preprocessor

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represented hair cell damage as dashed-line symbols (Santi et al., 1986), or in histogram plots (Clark and Bohne, 1978). In order to produce the cytocochleogram, the condition of each hair cell along the length of the basilar membrane must be determined. This is performed manually and is rather tedious and time consuming given that a mouse cochlea contains approximately 3000 hair cells. In addition to assessing the type of structural damage, the location of the structure must also be determined. We have previously described a computer-assisted method to morphometrically assess hair-cell damage and to produce a cytocochleogram (Santi et al., 1986). The system required a complete plastic-embedded surface preparation, a specialized microscope with a digitized stage, and a computer program to map cell structures onto an X, Y coordinate system. After the tissue segments were mapped, the computer determined the location of the cells relative to their percent distance along the basilar membrane. A second pass along the surface preparation was performed in order to record the condition of the hair cells. After recording all of the hair cell damage, the program then produced a graphic representation of hair-cell damage as a function of distance along the basilar membrane. The main drawback of this system was that it required a specialized microscope fitted with micrometers in order to analyze cochleas.

The Digital Cytocochleogram program that we describe in the present communication uses some of the programming methods from our previous work, but the present method does not require a specialized microscope to map and analyze the surface preparations. With the development of the Internet and high speed access of large files it is now feasible to provide complete surface preparations of the organ of Corti/basilar membrane complex, in the form of digital images, for any number of cochleas. One could simply make image files available for viewing or downloading. However, it would be more useful if users could interactively and morphometrically analyze these images over the Internet. This is was what has been accomplished with the development of the Digital Cytocochleogram resource of the mouse cochlea database (MCD). Thus, the term "Digital Cytocochleogram" described in this publication refers to an interactive program that one can use to view, count, and measure structures on digital images of the organ of Corti/basilar membrane complex of a mouse cochlea. The Digital Cytocochleogram resource of the MCD is available at the following URL: http// mousecochlea.ccgb.umn.edu.

2. Methods and results

Specimen preparation, digital imaging, and software tools used to produce the Digital Cytocochleogram are described below.

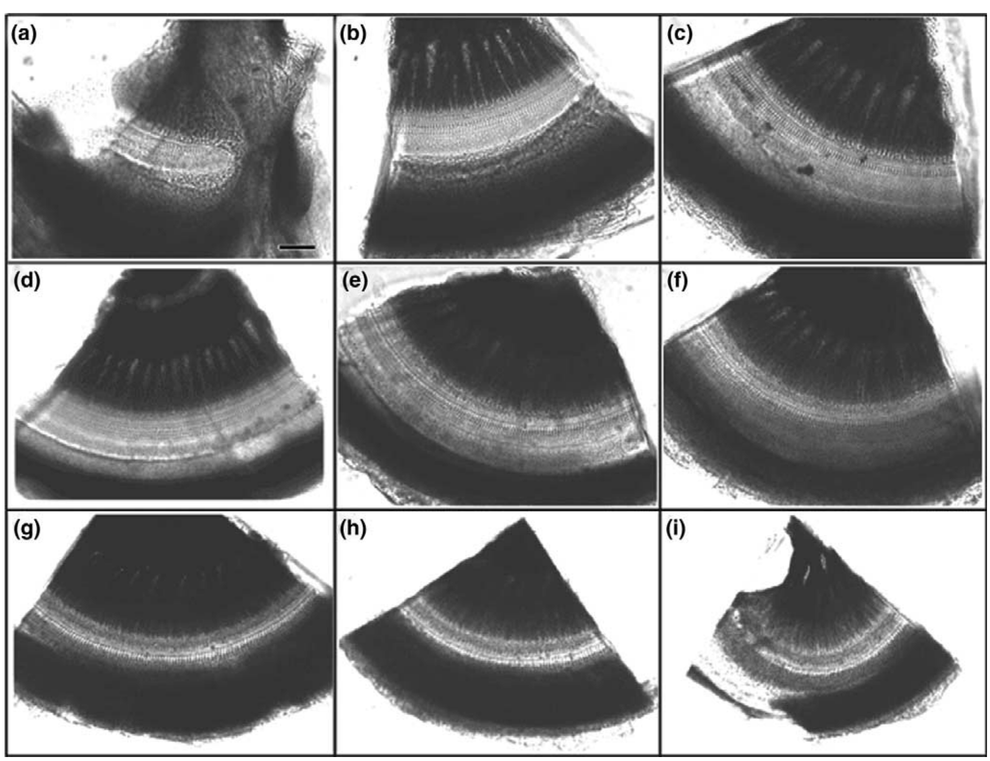

Fig. 1. A composite digital image showing all nine segments (panels a–i) of a complete surface preparation of a mouse cochlea. The images are arranged from the base to the apex of the cochlea and panel a contains a 100 μm magnification bar.

3. Whole-mount, surface preparations and digital imaging

The specimen of the mouse cochlea described in this publication was loaned to us for digital imaging by Dr. Barbara Bohne. The cochlea was from a wild-type mouse with a C57BL/6J background. It was fixed with a buffered solution of osmium tetroxide and processed as a plastic-embedded, surface preparation by and according to the procedure described by Bohne and Harding (1997). This complete specimen consisted of 9 tissue segments embedded in a thin plastic block. The block was mounted on a microscope slide for digital imaging. Each tissue segment was digitally photographed using an Olympus Vanox microscope, Nomarski illumination, and a Q-Imaging digital color camera (Fig. 1). In order to digitize the full size of each segment they were photographed at a relatively low magnification of 105× using a 10× Olympus objective. At this magnification hair cell stereocilia cannot be resolved. Digital images from each segment were stored on a Dell Windows computer and a micrometer slide was used to accurately determine the magnification of the images.

4. Mapping and measuring

We have previously used the arc-distance approximation method to determine the curved length of the basilar membrane (Santi et al., 1986). However, the present method differs substantially from our previous method in which a microscope with digitized X, Y axes was required. The present method only requires that digital images of the complete length and width of the organ of Corti/basilar membrane complex be obtained. An X, Y coordinate system was applied to each of the digital images, not the tissue segments as described in our previous publication. One restriction of the arc-approximation method is that the cochlear turn segment must not exceed 180° of arc. The process of mapping and then measuring structures along the organ of Corti can be divided into two parts, called Pass 1 and Pass 2. In Pass 1, the images are mapped to an X, Y coordinate system, and in Pass 2, the map is used to determine the location of a structure along the length of the basilar membrane. Pass 2 also measures the distance between any two points on the images relative to their location along the basilar membrane.

5. Pass 1: arc approximation and mapping

5.1. Digital Cytocochleogram coordinate finder program

To begin the Pass 1, all of the segments comprising a complete surface preparation of the organ of Corti along the length of the basilar membrane are digitally imaged and their final magnification is determined.

In order to determine the curved distance along the length of the basilar membrane, an arc is fitted to a trace line or reference location (i.e., lateral border of the inner pillar cell headplates) on each image using the following method. Three coordinate points (X_1, Y_1), (X_2, Y_2), (X_3, Y_3) are sampled at the beginning, middle, and end of each tissue segment image along the trace line (Fig. 2).

Next, straight line equations from points (X_1, Y_1), $(X_2, Y_2), (X_3, Y_3)$ are computed using the point-slope formula (1)

$$Y_2 - Y_1 = m(X_2 - X_1) \quad \text{where } m = \frac{(Y_2 - Y_1)}{(X_2 - X_1)}. \tag{1}$$

Two line equations, one extending from (X_1, Y_1) to (X_2, Y_2), and one extending from (X_1, Y_1) to (X_3, Y_3) are formed (2) and (3) and marked as L_1, and L_2 (Fig. 3). These equations are as follows:

$$L_1 = Y = Y_1 + m_1(X - X_1) \quad \text{where } m_1 = \frac{(Y_2 - Y_1)}{(X_2 - X_1)}, \tag{2}$$

$$L_2 = Y = Y_1 + m_2(X - X_1) \quad \text{where } m_2 = \frac{(Y_3 - Y_1)}{(X_3 - X_1)}. \tag{3}$$

Next, the perpendicular bisectors of lines L_1 and L_2 denoted on Fig. 3 as points (X_4, Y_4) and (X_0, Y_0) are found. These points are calculated using the following Eqs. (4) and (5).

$$X_0 = \frac{(X_1 + X_3)}{2} \quad \text{and} \quad Y_0 = \frac{(Y_1 + Y_3)}{2}, \tag{4}$$

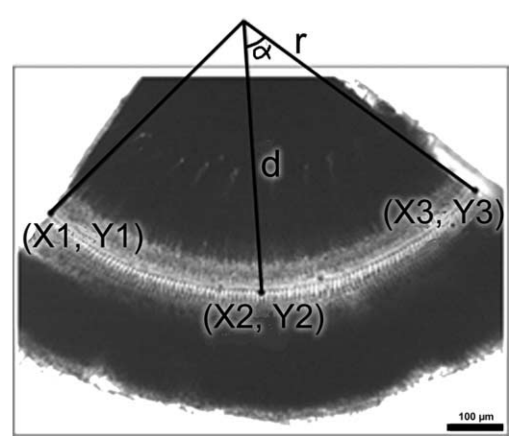

Fig. 2. The arc approximation method uses three coordinate points to determine the radius (r), chord length (d), and arc angle α of the segment. Points (X_1, Y_1) and (X_3, Y_3) are the endpoints of the segment, while (X_2, Y_2) is any point along the basilar membrane, through which the arc will pass. The arc approximating the length of the segment is constructed so as to pass through all three points.

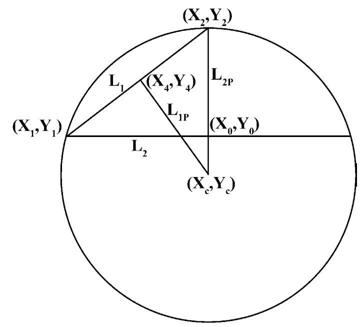

Fig. 3. This diagram shows a general case of the arc approximation method. Points (X_1, Y_1), (X_2, Y_2), and (X_3, Y_3) are points along the segment as in Fig. 2. Lines L_1 and L_2 are then constructed between (X_1, Y_1) and (X_2, Y_2), as well as (X_1, Y_1) and (X_3, Y_3). Perpendicular bisectors (L_{1P}, L_{2P}) of each line are then constructed from the mid-points (X_4, Y_4) and (X_0, Y_0) of each line, providing the center of the circle (X_C, Y_C) at the point where they intersect.

$$X_4 = \frac{(X_1 + X_2)}{2} \quad \text{and} \quad Y_4 = \frac{(Y_1 + Y_2)}{2}. \tag{5}$$

Next, the line equations of the perpendicular bisectors of L_1 and L_2 denoted as L_{1P} and L_{2P}(6,7) passing though points (X_4, Y_4) and (X_0, Y_0) are found:

$$L_{1P} = Y = Y_4 + \left(-\frac{1}{m_1}\right)(X - X_4), \tag{6}$$

$$L_{2P} = Y = Y_0 + \left(-\frac{1}{m_2}\right)(X - X_0). \tag{7}$$

The equations L_{1P} and L_{2P} are then solved simultaneously for X and Y to reveal their point of intersection (X_c, Y_c), the center of the circle (Fig. 3).

Once the center point (X_c, Y_c) is found, the radius, the arc angle, and the arc length are determined. The distance Eq. (8) is used to compute the radius (Figs. 2 and 4).

$$r = \sqrt{(X_1 - X_c)^2 + (Y_1 - Y_c)^2}. \tag{8}$$

The arc length, or distance of an arc extending along the trace line, is computed using the following derivation.

From (Fig. 2)

$$\sin(\alpha) = \frac{d}{2r}, \tag{9}$$

Rearranging,

$$\alpha = \sin^{-1}\left(\frac{d}{2r}\right), \tag{10}$$

where

$$\text{arclength} = r\theta \tag{11}$$

and

$$\theta = 2\alpha. \tag{12}$$

Combining (9)–(12),

$$\text{arclength} = 2r\sin^{-1}\left(\frac{d}{2r}\right). \tag{13}$$

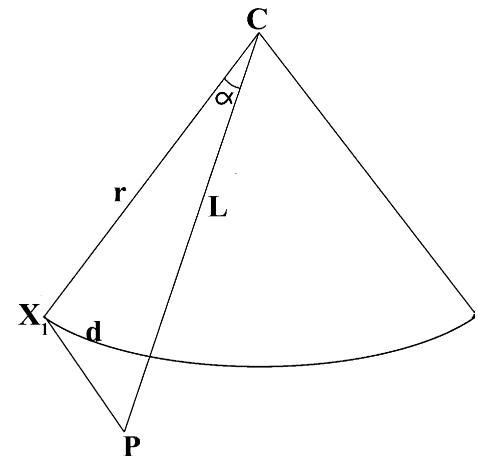

Fig. 4. The angle locations needed to calculate the percent distance from the apex. The line (L) between the mouse cursor position (P) and the center point of the circle (C) forms angle (α) with the line from the center point to the base point (X_1) of the segment. The arc lengths of the previous segments are summed and added to the arc distance (d) between the mouse cursor position (P) and the base point of the segment (X_1) along the trace line.

In Pass 1, all of the derivations and equations are programmed into the Coordinate Finder, a JavaScript application written to calculate arc length (Fig. 2). This program accepts three points (X_1, Y_1), (X_2, Y_2), and (X_3, Y_3) along the trace line for each segment and calculates the center point (X_c, Y_c), radius, arc length, and arc angle of that segment. This program is used only once when the segment image is initially mapped to an X, Y coordinate system. It is not necessary for Internet users of the MCD to use the Coordinate Finder program. However, this program will be available as a download from the MCD website if a user wishes to map their own surface preparation images.

Choosing different points for the middle (X_2, Y_2) point along the reference line shows very little change in the radius computed (Fig. 5). This provides an example of the robustness of the arc-approximation technique, allowing users to select any point along the reference line as the middle point and retaining confidence that the arc computed and the resulting distances are accurate.

The Coordinate Finder program was used to determine the length of the basilar membrane for each segment relative to the total length of the basilar membrane. In addition, precise distance along the length of the basilar membrane is displayed in real-time as the position of the screen cursor is moved by the mouse over the image of the segment. This is accomplished using JavaScript by capturing the X, Y position of the screen cursor, then calculating the cursor position angle (α) relative to the apex end of the basilar membrane (Fig. 6). To calculate the cursor position angle (α) relative to the base point of each cochlear segment (X_1), the law of cosines equation was used.

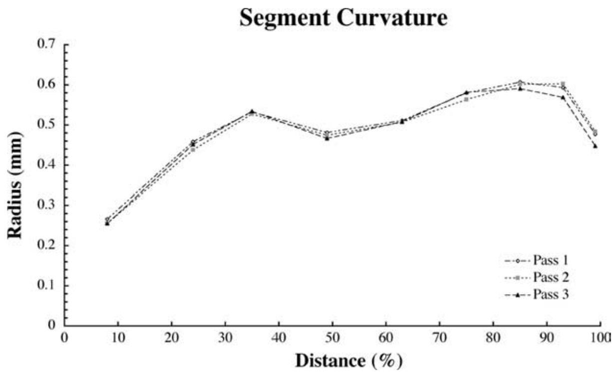

Fig. 5. A graph showing the curvature of each segment, as a function of percent distance along the length of the basilar membrane. The data points represent segments 1–9 from left to right as three separate passes along the length of the basilar membrane of the same cochlea. Each pass was made using the same points at the ends of the segments, but a different third point along the segment. The consistency of the data suggests that the arc approximation method is accurate regardless of the selection of the location of the middle point.

Law of cosines equation:

$$C^2 = A^2 + B^2 - 2AB\cos(\alpha). \tag{14}$$

Rearranging to solve for the cursor position angle (α)

$$\alpha = \cos^{-1}\left(\frac{C^2 - A^2 - B^2}{-2AB}\right). \tag{15}$$

The angle (α) is then used to compute the arc distance (d) of the screen cursor location. The arc distances from the previous segments are summed and added to the arc distance (d) to determine the percent distance from apex of the complete length of the basilar membrane (Fig. 4).

In addition to distance along the length of the basilar membrane the approximate frequency region of the structure is computed and displayed. This estimate is computed using the formula of Ou et al. (2000) reported for the mouse and is provided in the equation below.

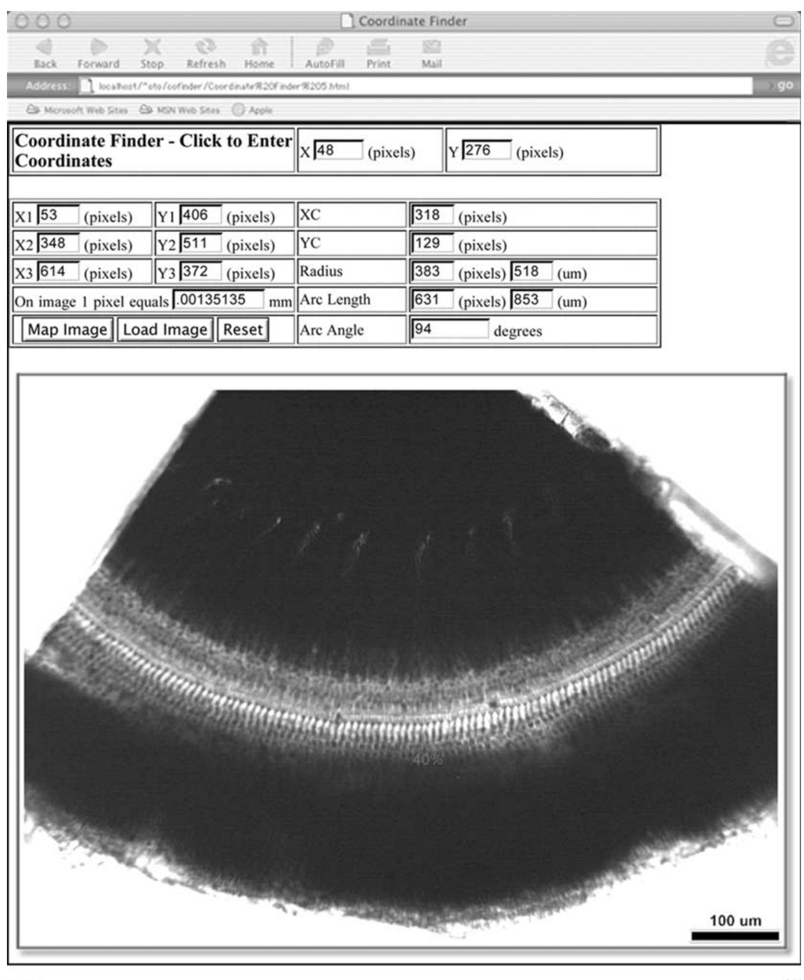

Fig. 6. The Digital Cytocochleogram Coordinate Finder program calculates a segment's arc length and arc angle after it receives three coordinate points along the trace line. These X, Y locations are recorded by a mouse click while positioning the screen cursor at the beginning, middle, and end point of the segment along the trace line. The user also must enter the conversion ratio from pixels to mm, which is determined from the final image magnification of the segment. After entering the necessary data, the user clicks on the ''Map Image'' button, which computes the center point (XC, YC), radius, arc length, and arc distance of the segment.

$$F = 1460(10^{0.0177x}) \text{ where } x \text{ is in percent distance.} \quad (16)$$

This process of determining distance is performed for all of the images that comprise the complete surface preparation. A web page is dynamically produced for each image, containing coordinate information and percent distance along the basilar membrane that the image contains, relative to the total length of the basilar membrane. The relatively low magnification required that only nine segment images be taken for the complete organ of Corti. Higher magnifications will require more digital images to cover the entire length of the basilar membrane.

In order to determine the accuracy of the Coordinate Finder program in computing basilar membrane length, the seventh segment from the apex was chosen and five trial measurements were computed using both a manual line segment fit method and the arc approximation method. Segment seven was chosen because of its uniform shape and clear trace line. These results were compared to Dr. Bohne's straight line-length estimate of basilar membrane length. (Table 1). The Coordinate Finder program estimated a mean basilar membrane length of the segment to be 0.744 mm. To perform a manual measurement, the digital image of segment was printed and point-to-point, straight line-length segments were drawn along the trace line using a ruler. Each line segment was 1.25 in. length, which equaled 100 μm of basilar membrane length at an image magnification of 105×. The line segments were counted and the length of any partial line segments were computed to provide the basilar membrane length using this manual measurement method. The line segment method yielded a mean basilar membrane length for segment seven of 0.744 mm. In addition, Dr. Bohne reported a total length of the basilar membrane for segment seven was 0.71 mm. It is not known which of these three methods is more accurate but they are within 4.6% of one another.

Next, the Coordinate Finder program was used to estimate the basilar membrane length of the entire mouse cochlea surface preparation. The complete length of the basilar membrane was 6.04 mm. (Table 2) To perform a manual measurement, all nine digital images were printed and point-to-point, straight line, length segments were drawn along the trace line. As with segment seven, each line segment was 1.25 in. length. The line segments were counted and the length of any partial line segments were computed to provide the total basilar membrane length using this manual measurement method. The line segment method yielded a basilar membrane length of 6.12 mm. Dr. Bohne reported a total length of the basilar membrane for this specimen as 6.00 mm. Again, it is not known which of the three methods is more accurate, but they are within 2% of one another. As an additional course check of distance for the online user, a mark has been drawn on the images at every 10% of the total distance of the length of the basilar membrane.

Table 1

Method	Mean segment length (mm), segment 7	SD
Bohne	0.710	–
Line segment	0.744	±.0038
Arc approx.	0.744	±.0046

Table 2

Method	Distance (mm) whole specimen
Bohne	6.00
Line segment	6.12
Arc approx.	6.03

6. Pass 2: structure distance and morphometry

6.1. Digital Cytocochleogram viewer

When a user accesses the Digital Cytocochleogram resource from a webpage on the MCD they will use the Viewer program. However, it is not necessary for a user to download or install any software to use the Viewer program as it uses client-side, JavaScript programming. Due to differences in the way web browsers perform page layout, small differences will appear in the position of the screen cursor on the page. However, since these differences may result in significant distance and frequency values when the cursor is moved across the digital images, we had to standardize which web browser would be compatible with the Digital Cytocochleogram. Microsoft Internet Explorer was chosen because it is available for both the Apple Macintosh and Microsoft Windows computers, and it is the most commonly used browser. However, users should check the MCD website to determine which versions have been certified to produce accurate results. The only other software requirement is that the web browser has JavaScript enabled.

The Viewer program (Fig. 7) allows a user to select which segment of the surface preparation to examine. Distance of each segment along the length of the basilar membrane (in percent) is displayed in the leftmost column. Clicking on the distance brings the segment image into the viewer window where it can be analyzed using the mouse cursor and button clicks.

Another feature of the Digital Cytocochleogram Viewer program is the straight-line measurement tool, which can be used to measure any structure on a digital image of a tissue segment (Fig. 7). The measurement tool was developed using JavaScript. The tool captures mouse clicks, calculates the distance between the two

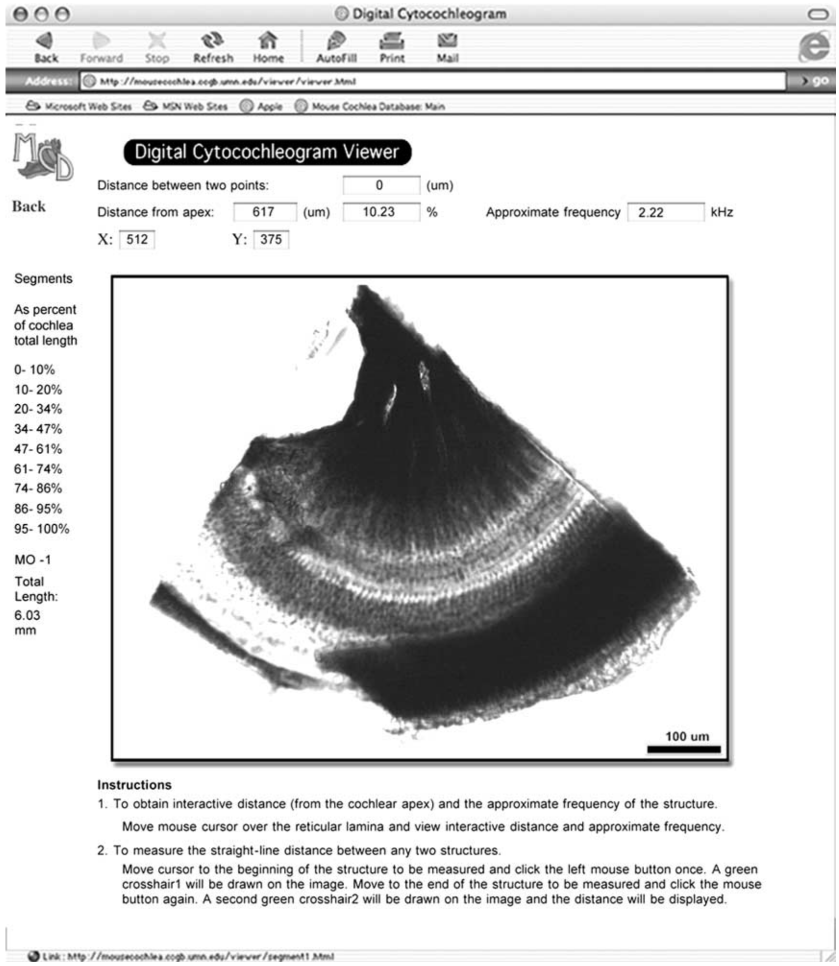

Fig. 7. The Digital Cytocochleogram Viewer program allows a user to select and measure any cochlear segment. In addition, the straight-line distance between any two points on the image can also be measured. By moving the mouse cursor over the tissue segment in the area around the basilar membrane, the user can determine the structure's position and approximate frequency along the length of the basilar membrane. This information is displayed in the text boxes at the top of the page.

clicks, and converts this distance from pixels to distance in micrometers. The tool captures mouse positions on each mouse click and places a digitized graphic of a crosshair so that each click can be located. A third mouse click or movement to another segment clears the length calculation between the last two points.

It is important to note that due to the way the arc is calculated that the entire web page in the Viewer program is mapped to an X, Y coordinate system. We attempted to restrict values to only the tissue segment, but that approach required extensive manual exclusion, which would be difficult for users who wish to use the Coordinate Finder program. It also decreased performance of the Viewer Program. A compromise was implemented so that the program returns values only in the image pane of the web page. However, a user must be aware that valid distance and frequency values are most accurate along the length of the trace line, and, in fact, erroneous values will be returned when the cursor is not over the tissue (Fig. 8).

7. Discussion

The Digital Cytocochleogram with the rest of the resources of the MCD is posted on the MCD website, currently located at http://mousecochlea.ccgb.umn.edu. It is anticipated that this URL will remain constant for some time since it is maintained on a University of Minnesota server. However, since URLs do change over time and this publication will not, users should be able to use an Internet search engine such as Google to locate the site using the keywords "Mouse Cochlea Database" or "Digital Cytocochleogram".

The Digital Cytocochleogram allows investigators to efficiently examine and measure structures on surface preparations of the organ of Corti/basilar membrane complex from one or more mouse cochleas. The value of providing this information over the Internet is primarily to provide a comprehensive collection of anatomical information on the mouse cochlea in a community accessible database. Presently the MCD contains images

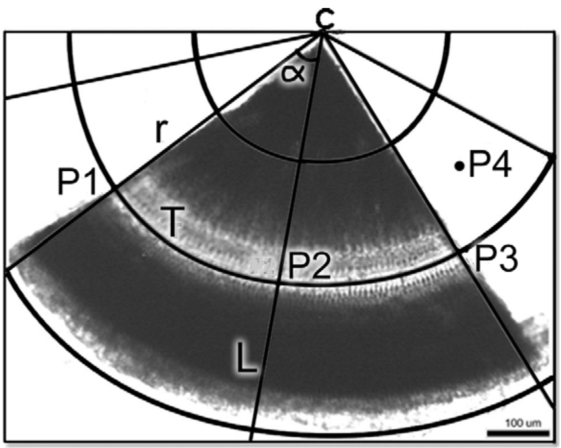

Fig. 8. This figure shows that a coordinate grid of points is computed and placed over the entire image plane of the Viewer program. Using the three coordinate points (P1, P2, P3) of the segment, a center point of the circle (C) is computed with angle (α) and the arc distance along the trace line (T) is computed and referenced to the other segments in the cochlea. Basilar membrane distance is only accurate along the trace line even though the program will compute distance from C along many lines B,L and even at point (P4), which is completely off of the tissue.

of the organ of Corti/basilar membrane complex from a complete, well preserved, and dissected surface preparation of a normal mouse cochlea. Users may use this cochlea to examine the normal anatomy of a mouse surface preparation, measure structures on the images, and determine the relationship between distance along the basilar membrane and approximate hearing frequency range in the mouse. This process can be performed over the Internet using a simple web browser.

It is anticipated that additional mouse cochleas from normal, experimentally altered, and mutant mouse strains will be added to the MCD. In addition, the Digital Cytocochleogram contains digital images of a surface preparation that are focused at the level of the reticular lamina. However, in the future, we would like to enhance the Digital Cytocochleogram in a number of ways. Tissue segments will be digitized at higher magnifications to allow for the visualization and measuring of structures at higher resolutions, like the hair cell stereocilia. In addition, higher magnifications will result in not all structures being in a single focus plane. Therefore, we will use software tools to extend the depth of focus to so that more structures will appear within the focal plane. Furthermore, segments will be digitized at different levels within the thickness of the organ of Corti. This will allow users to view and measure structures deeper than at the reticular lamina of the organ. Lastly, we plan to make it easier for users to record damaged hair cells using the Digital Cytocochleogram, and thus be able to plot and reconstruct a graphic depiction of hair cell damage along the length of the basilar membrane.

Acknowledgements

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