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Volumetric Imaging of Blood Flow within Cochlea in Gerbil *in vivo*

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Abstract

Changes in blood flow to the inner ear are thought to influence a number of cochlear diseases, including noise-induced hearing loss, sudden hearing loss, and Meniere's disease. Advances have been made in the areas of vital microscopic studies of micro-circulation, and the laser Doppler flowmetry. But none of these techniques can provide *in vivo* three-dimensional (3-D) mapping of microvascular perfusion within the cochlea. To overcome this limitation we have developed and used a method of optical microangiography (OMAG) that can generate 3-D angiograms within millimeter of tissue depths by analyzing the endogenous optical scattering signal obtained from an illuminated sample. We used OMAG to visualize the cochlear microcirculation of adult living gerbil through the intact cochlea, which would be difficult, if not impossible, by use of any other current techniques.

Index Terms

Cochlear blood flow; biomedical imaging; optical coherence tomography; optical microangiography

I. Introduction

The motivation behind the study of cochlear microcirculation is the supposition that it plays an important role in maintaining normal homeostatic environment in the inner ear and also by the assumption that some cochlear diseases are vascular in nature. It is hypothesized that these disorders can be treated by increasing blood flow to the inner ear. It is known that there is high metabolic demand of cochlear tissues such as stria vascularis[1,2], and that ischemia may have potential role in cochlear injury following noxious insults such as sound[3–5] or ototoxic drugs[6]. It is also possible that vascular changes can be part of etiology of Sudden Deafness Syndrome [7,8], and Meniere's disease [9,10].

There is a clear need for understanding of the mechanisms controlling the cochlear blood flow. The study of blood flow in cochlea has interested auditory investigators for centuries. There have been many research activities, with applications of various techniques to measure cochlear blood flow. Intra-vital microscopy [11,12] was used for the first direct observation of cochlear blood flow. Intra-vital microscopy allowed visualization of the major vascular beds of the lateral wall and the basilar membrane in the living animal [6]. With this technique vessel lumen diameter, changes in blood flow pattern, flow rate, can be assessed under resting conditions and with experimental manipulations. However, the technique requires the removal of otic capsule over the lateral wall vessels to permit direct visualization. Under the best of condition it is a very invasive procedure.

Microsphere measurements of cochlear blood flow were later introduced [13–15]. By assessing the number of microspheres trapped in the capillary bed of the inner ear after the injection of a known quantity of spheres and the acquisition of reference blood samples, microsphere technique permits a quantitative measurement of cochlear blood flow. The drawback is that it is a static measurement and only a few measurements can be performed within a short period of time. It is also invasive as the microspheres flow through the blood vessels and hence there could be the possibility of affecting the blood flow.

Laser Doppler flowmetry [16,17] is another technique that has been used to measure cochlear blood flow. This technique gives a voltage output which is proportional to the velocity of blood. The disadvantage of this technique is uncertainty of knowing the measurement volume. While it may be known that the measurement is confined within the cochlea, the vessels contained in the measurement cannot be confirmed.

There are also indirect measurements applied to measure cochlear blood flow in addition to these direct measurements. Micro-electrode oxygen tension determination [18–20] is one such measurement. Although this measurement provides good dynamic range and localized measurement, but it is only indirectly related to blood flow because both oxygen utilization and supply are simultaneous component of the measurement.

In this report we demonstrate a new technique of optical micro angiography (OMAG) that has the potential to solve many of the disadvantages of the techniques currently in use. OMAG can be used in an intact cochlea of a living specimen, without introduction of microspheres or removing of the otic capsule over the lateral wall vessels. Since, OMAG provides three dimensional visualization of the cochlea blood flow, it removes the uncertainty of measurement volume, enabling us to visualize the vasculatures of the cochlea and make localized measurements. OMAG provides non-invasive, high resolution, high sensitivity mapping of blood flow within thick tissue layers *in vivo*.

II. OMAG System Setup

The schematic diagram of the system used in this study to perform *in vivo* optical angiography is shown in Figure 1. It is based on the Michelson optical interferometer and is similar to a fiber-based optical angiography (OMAG) system previously reported [21,22]. The light source for the system is a superluminescent diode (SLD) with a central wavelength of 842 nm and FWHM bandwidth of 45 nm that gives axial resolution of $\sim 8\mu\text{m}$ in air. The light, after passing through an isolator, is coupled into a fiber based Michelson interferometer using a 2×2 coupler. In the reference arm light was reflected from a mirror that was mounted on a piezo-translation stage, which was driven by a 10 Hz saw tooth waveform with $50\mu\text{m}$ amplitude. The sample probe consisted of an X-Y galvanometer scanners and optics to deliver and collect backscattered light from the sample. The lateral resolution was $\sim 16\mu\text{m}$ determined by the objective lens used to focus the incident light on the sample. The incident light on the sample was $\sim 1\text{mW}$. The reflected light from the sample and reference arm was combined and sent to a custom built spectrometer. The spectrometer was made of a 30mm (focal length) collimating lens, a 1200 lines/mm diffraction grating and 150mm focal length focusing lens. The focused light spectrum was captured by a line scan CCD (Basler Vision Tech., Germany) consisting of 2048 pixels, each with 10×10 size, line rate of $\sim 29\text{kHz}$ and bit depth of 10 bits. Polarization controllers were used in the reference, sample and detection arm to maximize the contrast of the interference signal. The spectral resolution of the spectrometer was 0.055 nm, resulting in an optical ranging of 6.4mm in air, i.e., the full depth in the Fourier space. The positive frequency space (3.2 mm) was used for micro-structural imaging and negative space (3.2 mm) was used for flow imaging.

The probe beam was scanned in the lateral direction by the X scanner driven by a 10 Hz saw tooth waveform with equivalent amplitude of 2.2mm. The probe beam was scanned in the y-direction by Y scanner moving 2.2 mm at 0.02 Hz. The camera integration time was set at 99 μ s for imaging and allowing 1 μ s to download the spectral data from the CCD to the host computer using CameraLinkTM and a high speed frame grabber board (PCI 1428, National Instrument, USA). This means the CCD line rate was about 10 kHz. In the x-direction there were 1000 points, meaning a data matrix of 1000 $\times$ 2048 elements for each B-scan. In the y-direction there were discrete points for over 2.2 mm. Thus there were 1000 $\times$ 2048 $\times$ 500 elements in the data cube for the entire 3-D imaging. It took about 50s to obtain the data cube using the setup in Figure 1.

The key difference between OMAG and Fourier domain OCT (FDOCT) is that the spectral interferogram is modulated by a constant Doppler frequency that makes it feasible to separate the moving and static scattering centers in the sample. In the current setup this is achieved by moving the mirror in the reference arm by a piezo driven stage at a constant velocity during the B-scan (x-direction). The Doppler frequency can also be introduced by offsetting the beam from the pivot point of the scanner in the sample arm [23]. Other methods to introduce Doppler frequency involve for example introducing phase modulators in the reference arm or the sample arm or both[24–26].

III. Mathematical Perspectives and Data Processing

Optical interferometry has long been used to detect movements such as red blood cells movements or back ground tissue movements. The mathematical analysis of the OMAG method essentially maps the velocities moving into the tissue away from the surface into one image and velocities moving out of the tissue towards the surface into another image.

In case of the OMAG (in Figure 1), if we assume that the reference mirror mounted on the piezo-stage moves at a velocity $v \rightarrow_{ref}$, with the probe beam proceeding in the B-scan (x-direction) with a velocity v_x (scalar), and if the particle that is detected by OMAG is moving with a velocity $v \rightarrow_s$ projected in the beam direction, then for simplicity we can assume that the spectral interferogram in λ is given by,

$$B(1/\lambda, x) = \cos \left(\frac{4\pi \left(z_s + (\vec{v}_{ref} + \vec{v}_s) \frac{x}{v_x} \right)}{\lambda} + \phi(x, z, \lambda) \right) \quad (1)$$

where z_s is the initial depth position of the scattering particle at the lateral position x , and $v \rightarrow_s$ is the velocity of the scattering particle, such that, the path length difference between the sample arm and the reference arm is $2(z_s + (v \rightarrow_{ref} + v \rightarrow_s)t_x)$, where $t_x = x/v_x$ is the scanning time of the probe beam and factor 2 accounts for the fact that the light goes in and out of the sample. The term $\phi(x, z, \lambda)$ is the optical phase term that related to the heterogeneity of the sample in the x-direction. Hence we see that $B(1/\lambda, x)$ is a sinusoidal function in x for a given λ . Following the arguments previously reported [22]; the analytic function of the real function B can be constructed through the Hilbert transformation if the Bedrosian theorem holds [27, 28] (According to which if the frequency arising from the path length difference between the reference and the sample arm is different than the frequency arising from the phase fluctuation ϕ , then the Hilbert transformation of B is given by its quadrature representation). Hence, the analytic function of equation (1) is given by,

$$\begin{aligned}\tilde{H}(t_1, t_2) = & \cos(2\pi(f_m - f_D)t_2 + 2\pi f_0 t_1 + \phi) \\ & + j\sin(2\pi(f_m - f_D)t_2 + 2\pi f_0 t_1 + \phi)\end{aligned}\quad (2)$$

where $f_m - f_D > 0$. If $f_m - f_D < 0$, then,

$$\begin{aligned}\tilde{H}(t_1, t_2) = & \cos(2\pi(f_m - f_D)t_2 + 2\pi f_0 t_1 + \phi) \\ & - j\sin(2\pi(f_m - f_D)t_2 + 2\pi f_0 t_1 + \phi)\end{aligned}\quad (3)$$

If we take the FFT of the complex function described in equation (2) and equation (3) with respect to t_1 , we see that the frequency f_0 sits in the positive frequency space for equation (2) and negative frequency space for equation (3).

We can see from equation (4) that the velocities $v \rightarrow_{ref}$ and $v \rightarrow_s$ decide whether the analytic function of equation (1) constructed by Hilbert transform is turned into equation (2) or equation (3). The analytic function is sequentially constructed by taking Hilbert transformation along the x axis of the B-scan for each $1/\lambda$ position.

If $v \rightarrow_s = 0$, positive velocities ($v \rightarrow_{ref}$, towards the incident beam) will modulate the signal with positive frequencies and negative velocities (away from the incident beam) will modulate the signal with negative frequencies. The Hilbert transformation along the x-direction of the B-scan converts the modulated signal versus x into a complex function, $\tilde{H}(1/\lambda, x)$. Any subsequent FFT along $1/\lambda$ of the Hilbert encoded function, $\tilde{H}(1/\lambda, x)$, will map positive frequency components in positive frequency space and negative frequency component into negative frequency space. This is in contrast to taking FFT of $B(1/\lambda, x)$ along $1/\lambda$ direction, which maps both positive and negative frequencies in both positive and negative frequency space and effectively halving the depth of scan.

If $v \rightarrow_s \neq 0$, and the value of $v \rightarrow_s$ is large enough to change the sign of $v \rightarrow_{ref} + v \rightarrow_s$, then after the operation of Hilbert transformation and FFT the signal will map into frequency space opposite to that of the case of $v \rightarrow_s = 0$. Hence the signal from the moving blood cell can be separated from the static tissue in the frequency space of FFT.

In the current study the reference mirror was moved at $300\mu\text{m/s}$ towards the beam splitter, hence any blood cell moving towards the surface at a rate less than $300\mu\text{m/s}$ (as it will not reverse the sign of $v \rightarrow_{ref} + v \rightarrow_s$) will not be mapped into the blood perfusion image but will be mapped into the static image. Only blood cells moving at a rate higher than $300\mu\text{m/s}$ towards the surface will get mapped into the blood perfusion image. In order to get the blood perfusion away from the surface the reference mirror is moved away from the beam splitter. The detailed data processing algorithm is described in reference [24].

IV. In Vivo Imaging of Gerbil Cochlea

In order to demonstrate the potential for using OMAG for studying *in vivo* cochlear blood flow and imaging the cochlear microstructures for small animal models, we obtained detail cochlea blood perfusion images of gerbil. Gerbil was chosen as our animal model for its relatively thin bony otic capsule. The experimental protocol was in compliance with the Federal guidelines for care and handling of small rodent and approved by the Oregon Health & Science University Institutional Animal Care and Use Committee. The middle ears of two deeply anesthetized gerbils were surgically exposed to provide a window for OMAG imaging. The surface of the cochlea was wiped with a cotton pledget to remove the periosteum vessels. The animals were

wrapped in a heating pad which was thermoregulated to 38.5°C. The animals were then placed under the OMAG scanning probe as described above and full 3-D scans of the cochlea of the prepared animals were then taken.

The animal preparation provided us with a wide optical view for OMAG to image. Figure 2 shows *in vivo* 3D OMAG image of gerbil cochlea. The imaging volume was 2mm × 2mm × 2mm. Figure 2(A) shows the image of the microstructures within the imaged tissue volume. In Figure 2(B) 3D OMAG image of the blood vessels in the bone and in the tissue just inside the bony otic capsule is shown. Figure 2(C) is the 2D projection of the 3D image in Figure 2 (B). In the projection image one can see the blood vessel network, including the capillaries, in the first turn of the cochlea.

Figure 3 shows the cross-sectional image of the cochlea. It combines the structural image and the flow image. In Figure 3(A), a midmodiolar section (x-z) of a living gerbil cochlea is shown. The otic capsule, the organ corti are identified in the Figure. Figure 3(B) and 3(C) shows an x-y cross-section image of the positions marked, B and C, in Figure 3(A) respectively. It is interesting to note that the majority of blood flow is in the tissue of the cochlear lateral wall (the spiral ligament and the stria vascularis). These images suggest the feasibility of using OMAG to monitor and image blood flow in cochlea with intact otic capsule.

V. Discussion

Although Figures (2) and (3) shows the promise of using OMAG to image the cochlear microstructures and cochlear blood flow in the lateral wall through the intact otic capsule, it may have difficulty imaging the flow in the osseous spiral lamina, a double plate of bone winding spirally around the modiolus. The difficulty may be because of the attenuation of light by the bony otic capsule. In order to overcome this problem, the solutions could be: 1) to increase the power of the probing light signal so the light backscattered by the deep tissue can be enhanced. 2), The probing wavelength in this study was 842 nm. It is known that the optical attenuation in the biological tissue is wavelength dependent. One study has shown that a light with longer wavelength (e.g., 1310 nm) increases the imaging depth for OMAG as compared to 800 nm wavelength band [29]. Thus, optimization of the optical wavelength used is necessary in order to enhance the imaging depth in the cochlea for OMAG. 3), The OMAG can image the osseous spiral lamina through the transparent round window membrane. The round window is situated within a funnel-shaped depression (the round window niche) and opens into the scala tympani of the cochlea. Because of minimal light attenuation, the round window provides an excellent niche for OMAG to image that part of the vascular network in the osseous spiral lamina or larger vessels that are more proximal to the spiral modiolar artery and vein. To determine the feasibility of imaging cochlear blood flow through the round window membrane, we have imaged gerbil cochlea through round window membrane. Figure 4 shows a typical image of a larger vessel that the OMAG was able to measure through the round window membrane with its current low power (~1mW). The image shows the merged flow and volumetric rendering of the tissue morphology, demonstrating the feasibility of using OMAG in the small animal model.

VI. Conclusion

We have demonstrated the feasibility of using optical micro angiography technique to obtain *in vivo* high resolution images of cochlear microstructures and cochlear blood flow with an intact otic capsule. By using the inherent properties of optical coherence tomography signals OMAG was able to separate the static parts from the moving parts and obtain high resolution blood perfusion images embedded in cochlear microstructures. OMAG provides a new technique to study cochlear blood circulation, without the limitations of currently used

techniques. Unlike, intra-vital microscopy, OMAG can be performed with an intact otic capsule. OMAG is less invasive than microsphere method. OMAG has the ability to provide localized measurements which laser Doppler flowmetry cannot.

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Figure 1.

The setup for the OMAG system used to perform 3-D angiogram from the 3D interferogram collected by it. To obtain a 3-D scan, the sample was scanned by the x-y galvanometer scanner with x-direction being the preferential direction of scanning. P.C.: polarization controller, CCD: charge coupled device.

Figure 2.

OMAG image of the gerbil cochlea in 3-D. Image volume is $2.0 \times 2.0 \times 2.0 \text{ mm}^3$. (A) Ricky could we call this just a 'surface image'? image of microstructures within the scanned tissue, where the location of the cochlea is indicated by the dashed outline. (B) Corresponding volumetric image of the blood perfusion within the cochlea. (C) 2-D projection view (x-z view) of blood flow (B) in the cochlea; the image shows the blood vessel network, including capillaries, in the first turn of the cochlea

Figure 3.

Merged view of the cochlear microstructure (gray scale coding) and blood flow (red-color coding) captured by OMAG in vivo. (A) A 'midmodiolar' section (x-z) of the living gerbil cochlea showing the otic capsule and the fluid and tissue within the cochlea. (B) and (C) sections (x-y) at the locations marked in (A), respectively. OMAG images provided flow information from capillaries in the lateral wall. White bar = 500μm.

Figure 4. OMAG imaging of cochlea in gerbils through the round window in vivo. The imaged tissue volume = $2.0 \times 2.0 \times 2.0 \text{ mm}^3$ (x-y-z). OMAG probe beam was directed from the top