OCT for non-destructive examination of the internal biological structures of mosquito specimen

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***Abstract*—The Study of mosquitoes and their behavioral analysis are of crucial importance to control the alarmingly increasing mosquito-borne diseases. Conventional imaging techniques use either dissection, exogenous contrast agents. Non-destructive imaging techniques, like x-ray and microcomputed tomography uses ionizing radiations. Hence, a non-destructive and real-time imaging technique which can obtain high resolution images to study the anatomical features of mosquito specimen can greatly aid researchers for mosquito studies. In this study, the three-dimensional imaging capabilities of optical coherence tomography (OCT) for structural analysis of *Anopheles sinensis* mosquitoes has been demonstrated. The anatomical features of *An. sinensis* head, thorax, and abdomen regions along with internal morphological structures like foregut, midgut, and hindgut were studied using OCT imaging. Two-dimensional (2D) and three-dimensional (3D) OCT images along with histology images were helpful for the anatomical analysis of the mosquito specimens. From the concurred results and by exhibiting this as an initial study, the applicability of OCT in future entomological researches related to mosquitoes and changes in its anatomical structure is demonstrated.**

Keywords—optical coherence tomography, Anopheles sinensis, non-destructive imaging, internal morphological analysis.

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I. INTRODUCTION

These mosquitoes are recognized as one of the major vectors of tropical diseases such as malaria, dengue, chikungunya, West Nile virus, yellow fever, lymphatic filariasis, Japanese encephalitis, Zika fever, and many other blood-transmittable diseases [1]. Approximately 212 million cases of malaria occurred in 2015, resulting in the death of approximately 429 000 people [2]. By recent findings, it has been concluded that Zika virus infection in pregnancy can be a cause of microcephaly [3]. Pathogens causing these diseases are transmitted from a mosquito to a host during blood feeding. Thus, research on mosquitoes, their feeding habits [4], probing behavior [5], life cycle [6], and the associated pathogen transmission plays a major role.

To date, many techniques such as micro-computed tomography [7], microscopic and histological analysis [8, 9], and X-ray imaging [10] have been implemented for mosquito studies. However, these techniques require time-consuming sectioning processes, a complex system design, or a long imaging acquisition time. Moreover, the cost of these techniques may be prohibitive for frequent implementation. Thus, a rapid bio-imaging technique would be of considerable advantage to researchers.

Optical coherence tomography (OCT) is a non-destructive imaging modality, which acquires cross-sectional images in real-time [11]. Applications of OCT have been demonstrated in various field of research, including ophthalmology [12, 13], otorhinolaryngology [14, 15], industrial inspection [16], agronomical studies [17-19]. Recently, OCT has been applied in various entomological studies, such as those developing neural morphological analysis and cardiac dynamics in Xenopus laevis [20, 21], age estimation of Calliphora vicina pupae [22], and arrhythmia caused by a Drosophila Tropomyosin mutation [23]. However, there have been no reports on the use of OCT as a tool for studying the internal organs of mosquito specimens.

In this study, we undertook a morphological analysis of *Anopheles sinensis* mosquitoes using spectral domain OCT (SD-OCT). Thus, we have demonstrated the potential application of OCT for identification of internal morphology in mosquito specimens. This was a feasibility study and by analyzing the results of two-dimensional and three-dimensional OCT images using histological images, we have demonstrated the advantages of using OCT for analysis of mosquito organs and tissue structures for future studies.

II. Materials And Methods

A. *Preperation of Mosquito Specimen for Experiment*

Mosquito samples were collected in 2014 at Majeong-ri, Paju-si (37°52′53.52″N, 126°45′24.89″E), Republic of Korea, using a black light trap. Specimens were identified using the molecular method described by [24] (the methods used and the detailed explanation of the procedures followed for identification of specimen has been explained in previously published article). After DNA extraction from a single leg using a genomic DNA extraction kit (Bioneer, Korea). The PCR conditions were as follows: The 12.5-µL PCR reaction mixture contained 0.5 µL genomic DNA of an individual mosquito, 0.5 units of Taq polymerase, 0.4 µM of each primer (see [24] for primer information), 1.5 mM MgCl2, 0.2 mM of each dNTP, and 1 X PCR buffer. The PCR cycling conditions were as follows: initial denaturation at 94 °C for 3 min, followed by 30 cycles at 94 °C for 30 sec, 55 °C for 30 sec, 72 °C for 2 min, and a final extension at 72 °C for 7 min. The PCR products were electrophoresed and visualized using ethidium bromide 2.5% agarose gel and a gel imaging system.

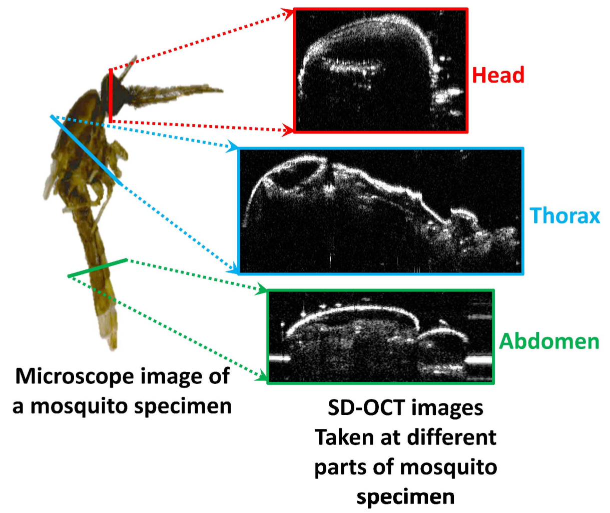

Figure 1. Microscopic and SD-OCT image of mosquito. Representative microscopic photograph of a mosquito specimen and its corresponding SD-OCT images taken at different parts of mosquito specimen.

A representative image of a mosquito specimen is shown in figure 1. After being anesthetized, specimens were carefully separated and stored. The experimental environment was maintained at 23 °C and 50% humidity. OCT imaging was carried out immediately after the specimens were anesthetized. For experimental convenience, the wings and the legs were clipped carefully without damaging other parts of the specimen.

B. *Specimen Preperation For Histological Analysis*

The specimens were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer for 24 hours under vacuum. Thereafter, the specimens were washed in sterile distilled water and dehydrated in a graded series of absolute ethanol (30%, 50%, 70%, 80%, and 90%) for 30 min. These steps are considered as gold standard methods for histological sectioning analysis [25]. These dehydrated specimens were infiltrated with propylene oxide and then embedded in Spurr's resin. The resin was polymerized in a dry oven at 80℃ for 7hrs. Finally, the samples were accurately sectioned using an ultra-microtome (MT-7000; RMC, Tucson, AZ, USA) and, stained with 2% methylene blue and observed under a light microscope.

C. *SD-OCT System Setup*

The OCT system was operated with a broadband light source with a center wavelength of 860 nm and a bandwidth of 165 nm. Objective lenses (10X) were used in reference and sample arms to improve the lateral resolution. The axial and lateral resolution of the OCT were 4 and 10 µm. The detailed configurations of the OCT instrumentation are provided elsewhere [19]. The schematic setup of the overall system is shown in figure 2.

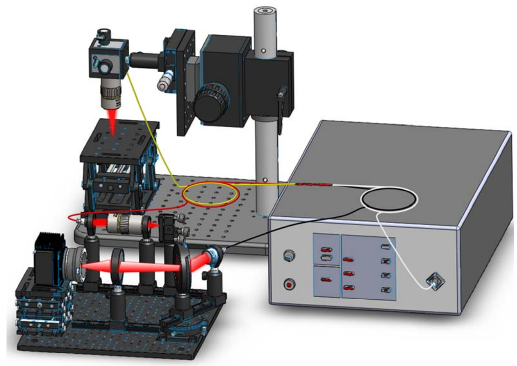
Figure 2. SD-OCT system setup used for the experiment.

III. Results And Discussion

A. *Three Dimensonal Volumetric Image Analysis*

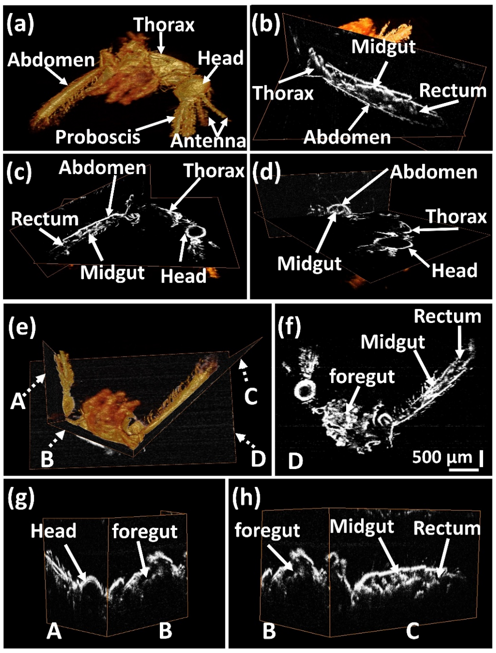

Figure 3. 3D OCT image of a mosquito specimen, and the orthogonal image section planes 1,2, 3, and 4 showing the continuity of the internal structures in the mosquito specimen. Refer to the supplementary media file for internal structure analysis at different depths.

Cross-sectional OCT images were acquired from the head, thorax, and abdominal regions of a mosquito body. Tomograms of the mosquito internal organs were studied by obtaining adjacent 2D-OCT images of all three regions. Using these prominent cross-sectional images, the 3D OCT images of these internal structures were rendered by acquiring adjacent 2D-OCT images and combining these images using volume-rendering software.

The volumetric OCT image is shown in figure 3(a). Figures 3(b), 3(c), and 3(d) show the orthogonal-sectioned images of the 3D images, revealing the internal morphology of head, thorax, and abdominal regions. The midgut and hindgut are shown in figures 3(c) and 3(d). In addition, individual sections of the head, thorax, and abdominal regions are shown in figure 3(e) using orthogonal sectioning planes, which are represented by dotted arrows with representations plane-1, plane-2, plane-3, and plane-4. The foregut, midgut, and hindgut are clearly identifiable through the individually sectioned images. The orthogonal image section planes-1, 2, and 3 helps to visualize the continuity of the internal structures in the specimen, showing the entire cross-section for easier analysis

B. Comparison Of Histology Images With OCT Images

For comparison of OCT images with the obtained histological images, 3D volumetric images were orthogonally sliced at different depths using post-image analysis software, the most correlative OCT image was selected for histological comparison. Figures 4(a) and 4(b) emphasize the comparison between OCT and histology of head and thorax regions. Foregut, head, proboscis, compound eye, and other internal structures in the thorax region are visualized in the OCT image, which are consistent with the histological image. The midgut and hindgut along with other internal abdominal structures are visible in the OCT image, was also found to be in correlation with the obtained histological image.

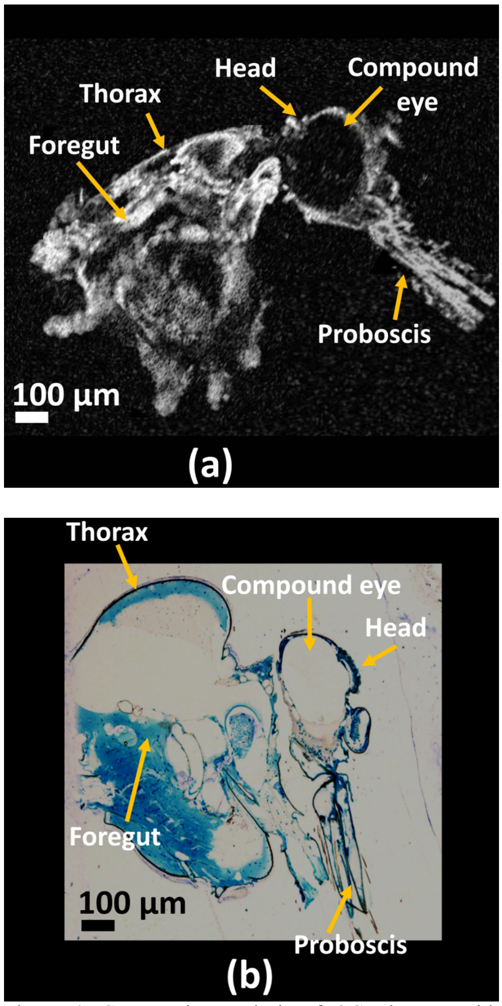

Figure 4. Comparative analysis of OCT images with histological images of a mosquito specimen. (a) *en face* OCT image, (b) is the microscope image of histology slice of mosquito specimen.

IV. Conclusion

We have demonstrated the benefits of three-dimensional imaging capability of OCT for structural analysis of *An. sinensis* mosquitoes. Internal structures, such as foregut, midgut, and hindgut, were clearly visualized using a customized OCT system supported by histological image verifications. To date, initial morphological studies and analysis of the anatomical structures of mosquitoes using OCT have not been performed. The results of the present study may represent a significant contribution and future in vivo studies on different mosquito vectors of disease-causing pathogens using OCT may help researchers to better understand how diseases are transmitted via mosquito bites. Future experiments can be focused on improving the resolution of the investigating OCT system by increasing the bandwidth of the laser source and detector, by improving the optical transmission of the OCT system for efficient signal detection [26-28] and for reduction of noise which may improve system sensitivity.

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