EQUALLY SLOPED X-RAY MICROTOMOGRAPHY OF LIVING INSECTS WITH LOW RADIATION DOSE AND IMPROVED RESOLUTION CAPABILITY

Shengkun Yao[1], Jiadong Fan[1], Yunbing Zong[2], You He[3], Guangzhao Zhou[3], Zhibin Sun[2], Jianhua Zhang[2], Qingjie Huang[4], Tiqiao Xiao[3], & Huaidong Jiang[*1, 2]

[1]School of Physical Science and Technology, ShanghaiTech University, Shanghai 201210, China
[2] State Key Laboratory of Crystal Materials, Shandong University, Jinan 250100, China
[3] Shanghai Synchrotron Radiation Facility, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China.
[4] School of Information Science and Engineering, Shandong University, Jinan 250100, China

Keywords: x-ray tomography, radiation dose, phase contrast imaging, equally sloped tomography

Summary: We present microtomography of a living specimen combining phase-contrast imaging and a Fourier-based iterative algorithm termed equally sloped tomography. Non-destructive 3D imaging of an anesthetized living yellow mealworm *Tenebrio molitor* was demonstrated with relatively low dose using synchrotron generated X-rays. Branching tracheoles and different tissues of the insect in a natural state were identified and analyzed.

1. INTRODUCTION

Determination of three-dimensional (3D) structural information of live organisms has advanced the understanding of many functions and evolutionary characteristics. Conventionally, internal structures of organisms such as insects are attained by histotomy in which the whole specimen is dissected into histological serial sections and investigated by light microscopy or electron microscopy. However, histotomy is a destructive, highly technical, labor-intensive method and it is much more desirable to investigate intact, living samples when possible. Non-invasive techniques such as confocal microscopy, magnetic resonance imaging (MRI), and ultrasound imaging are useful however they are limited by sample size and/or image resolution. With this in mind, developing non-destructive imaging techniques which can perform 3D structural analysis of living specimens is imperative.

X-rays provide an approach for imaging intact specimens with micron resolution because of their short wavelength and long penetration depth. Conventional X-ray radiography is based on differences in the absorption characteristics of the materials being imaged and has a long history of use in biology and medicine. Nevertheless, differences in absorption characteristics between different living biological tissues are quite small, which often leads to very low contrast and poor spatial resolution in the acquired images. Alternatively, phase-contrast X-ray imaging presents as a promising choice for enhancing the image resolution of biological specimens. Phase-contrast imaging is based on the phase shift of X-rays and significantly improves the resolution capability compared with absorption-based radiography. Recently, various phase-contrast imaging techniques have been developed including propagation-based imaging (i.e. in-line phase-contrast imaging), X-ray interferometry, grating-based imaging, and analyzer-based imaging. These imaging techniques have been widely used in biological applications, such as the investigation of millimeter-sized insects. However, 3D phase-contrast X-ray imaging of living insects is challenging due to the radiosensitivity of biological specimens. One efficient way to decrease the radiation dose to the specimens is reducing exposure time or projections. Thus, a powerful reconstructive algorithm plays an important role in low dose micro-CT research. Recently, numerous iterative tomographic algorithms such as model-based iterative reconstruction, compressed sensing, and penalized maximum likelihood have been developed to reduce or eliminate damage caused by the irradiation of a sensitive specimen.

In this letter, we present the 3D non-destructive imaging of living insects by combining phase-contrast X-ray microscopy and a powerful Fourier-based iterative algorithm termed equally sloped tomography (EST). We firstly verified the high performance of this algorithm compared with conventional filtered back projection (FBP) by

* e-mail: jianghd@shanghaitech.edu.cn

experimental reconstruction of biological samples. Then, a living yellow mealworm Tenebrio molitor was imaged based on phase-contrast X-ray technology and reconstructed with the EST algorithm from a very small number of projections.

2. EXPERIMENTAL METHOD

In-line phase contrast X-ray images were acquired with the beamline BL13W1 at the Shanghai Synchrotron Radiation Facility (SSRF). A collimated 12 keV X-ray beam was monochromatized by a double Si (111) crystal system. A mechanical shutter was placed in front of the sample to avoid radiation exposure in the absence of image capture. When the X-ray beam traverses the sample, the exiting beam carries both absorption and phase shift information. After propagating a sufficient distance, the phase shifts can be transformed into measurable intensity variations by Fresnel diffraction. A CCD detector with 2048 × 2048 pixels (pixel size of 9×9 μm) was used to transform the beam into an image. The specimen was fixed on a rotary stage that was strictly calibrated to parallel the CCD camera, and rotated 180° during data acquisition. Equally angled and equally sloped projections were acquired for FBP and EST, respectively. Furthermore, white field images, with light but no sample were acquired at ninety projections, and five dark field images without light in the light-beam station were acquired after data acquisition. The series of tilted projections were subsequently reconstructed by the FBP and EST algorithms.

3. RESULTS

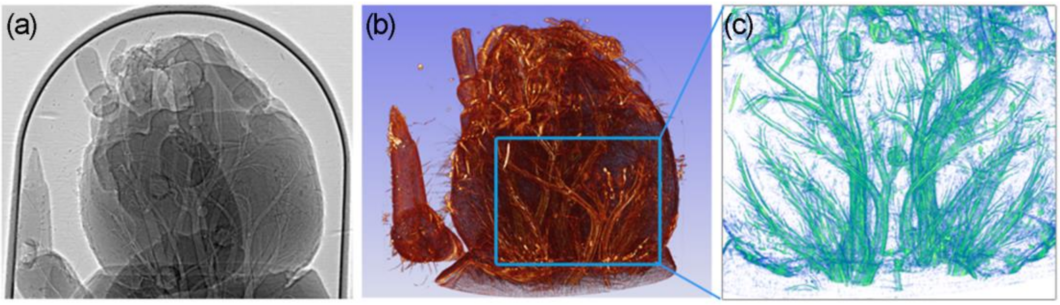

FIG. 1. In-line phase contrast images of a living Tenebrio molitor without phase retrieval. (a) A representative projection. (b) 3D microstructure of the Tenebrio molitor directly reconstructed by EST algorithm with 320 projections. (c) Branching internal tracheas and tracheoles. The intensity in (a) corresponds to the second derivative of the refractive index, showing the effect of edge enhancement, by which the branching tracheas and tracheoles can be visualized after tomographic reconstruction as shown in (b, c).

References

1. S. K. Yao, J. D. Fan, Y. B. Zong, Y. He, G. Z. Zhou, Z. B. Sun, J. H. Zhang, Q. J. Huang, T. Q. Xiao and H. D. Jiang, Applied Physics Letters, 2016, 108, 123702.
2. J. W. Miao, F. Forster and O. Levi, Physical Review B, 2005, 72.
3. H. Xie, B. Deng, G. Du, Y. Fu, Y. He, H. Guo, G. Peng, Y. Xue, G. Zhou, Y. Ren, Y. Wang, R. Chen, Y. Tong and T. Xiao, Journal of Instrumentation, 2013, 8, C08003-C08003.