Evaluating the Advantage of X-ray Microtomography in Microanatomical Studies of Small Arthropods

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Introduction

Conducting anatomical research on biological samples in sizes that range over a few millimeters is usually extremely time consuming. Such studies are typically done by analyzing histological section series. Mechanical artifacts caused by the cutting process mostly hinder the automatization of the 3-D reconstruction. Computer-aided reconstructions of histological data may produce exellent visualizations [1], but the time expended is a number of months per specimen. X-ray microtomography seems to be a promising alternative, since mechanical artifacts could be completely avoided. The time for data acquisition has recently become rather short because of the high brilliance of the radiation provided by third-generation synchrotron sources like the APS [2]. The goal of the recent study was to compare the quality of data from x-ray microtomography with that of histological data and evaluate its advantage for the field of work mentioned above.

Methods and Materials

A straight-ray projection microtomographic system as described in Reference 2] was used at the APS 2-BM beamline. The x-ray energy was varied from 6.0 to 12.0 keV by using a multilayer monochromator. A $CdWO_4$ ($10.0 \times 10.0 \times 0.5$ mm) scintillator screen was used. A set of Zeiss AXIOPLAN (5×, 10×, 20×) microscopic objective lenses was used. The charge-coupled device (CCD) camera was a peltier cooled MicroImager II (QImaging). A total of 720 projections measuring 1024×1024 pixels were taken; the samples were rotated around 180° in increments of 0.25° by a microstep rotary stage. The acquisition time was 0.5 s per projection.

Black field (x-ray beam shutter closed) and white field (beam without sample) were taken in steps of 20 projections. The data size was reduced to 512×512 pixels per projection after normalization. A set of 512 tomographic sections was reconstructed by a filtered back-projection algorithm by using the massive parallel linux-cluster available in sector 2 of the APS. 3-D visualization and segmentation were done by using the PC software package VGStudioMax (Volume Graphics).

The samples, fixed in 5% glutaraldehyd and stored in 70% ethanol, were scanned in wet condition by using ethanol-filled Kapton® capillaries and air-dried from hexamethydisilane (HMDS).

Results

An x-ray energy of 7.5 keV was used in all samples shown herein. The unprocessed projections (Fig. 1) already show an impressive number of anatomical details, which would not be visible by a light microscope. Browsing through sequences of subsequent projections might give a first impression of the spatial arrangement of some anatomical structures.

Regarding sample preparation, Fig. 2 clearly shows that the contrast is better in the dried specimen (Fig. 2b) than in the one scanned in wet condition (Fig. 2a), albeit drying artifacts are obvious here (i.e., collapsed gut).

The maximum resolution can be estimated in the ostracod crustacean by using a 20× objective (Fig. 3). Some massive muscle bundles obviously show myostriation (Z-band distance of about 2.5 µm). Therefore, the resolution achieved here was around 1 µm.

By comparing the quality of the tomographic data to that of histological sections, we notice that almost all

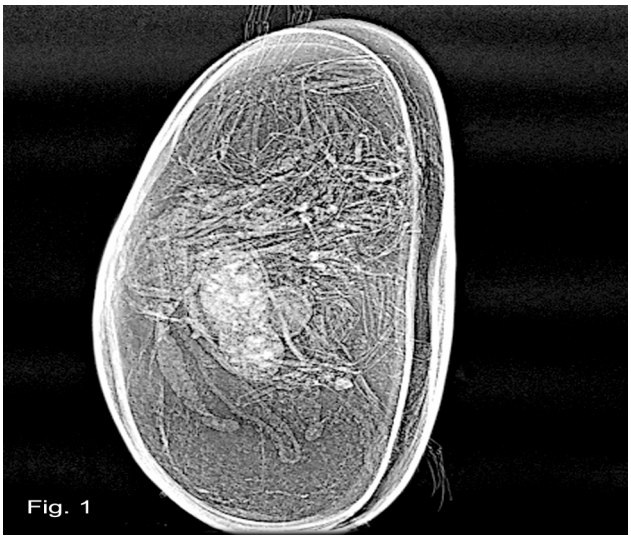

FIG. 1. Normalized projection of an ostracod crustacean specimen of approximately 0.8-mm total length.

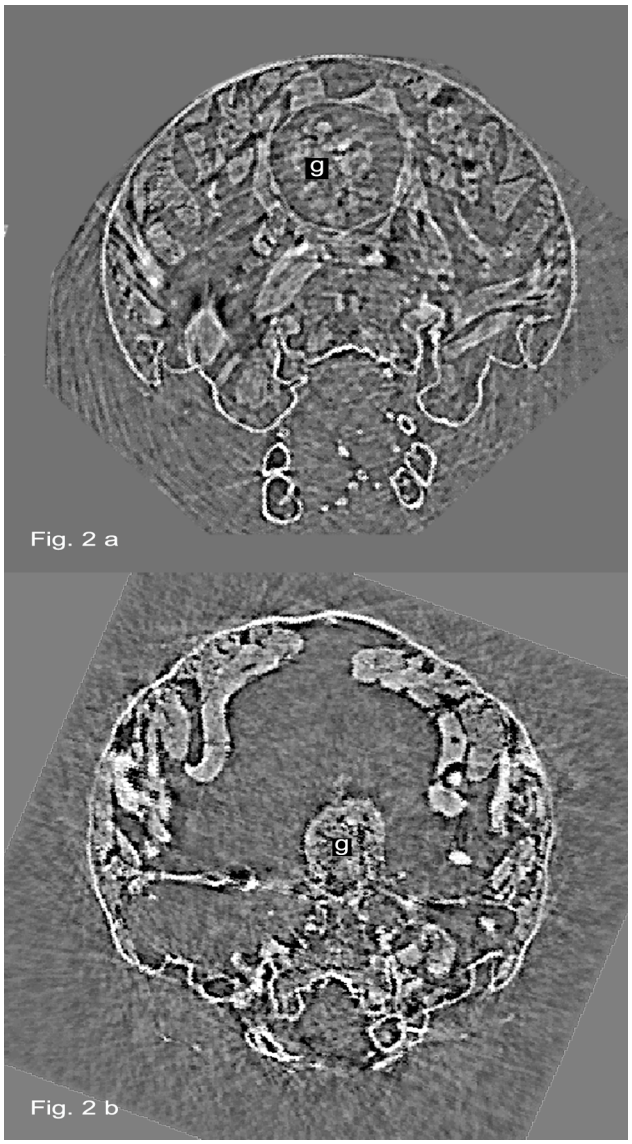

FIG. 2. Tomographic sections of the copepod crustacean specimen Cyclops sp. (sample length of 1 mm) scanned in wet (a) and dried condition (b).

tissues shown by histology are clearly discernible in the tomographic data (Fig. 4a and 4b) too.

In most cases, a grayscale segmentation of hard structures like the (calcified) cuticle and soft tissues (all remaining structures) can easily be done (Fig. 5a).

Discussion

Since the acquisition time was 0.5 s per projection, the whole data set might, in principle, be acquired in less than 10 min. (Solvable) technical problems raised the total acquisition time to about 1 h in practice. In addition, 15 min were needed for data processing, a time that might

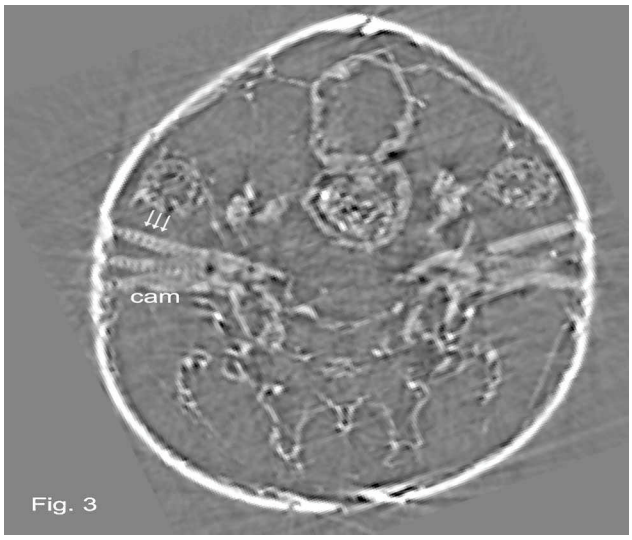

FIG. 3. Ostracod specimen, showing muscular striations in carapace adductor muscle (cam).

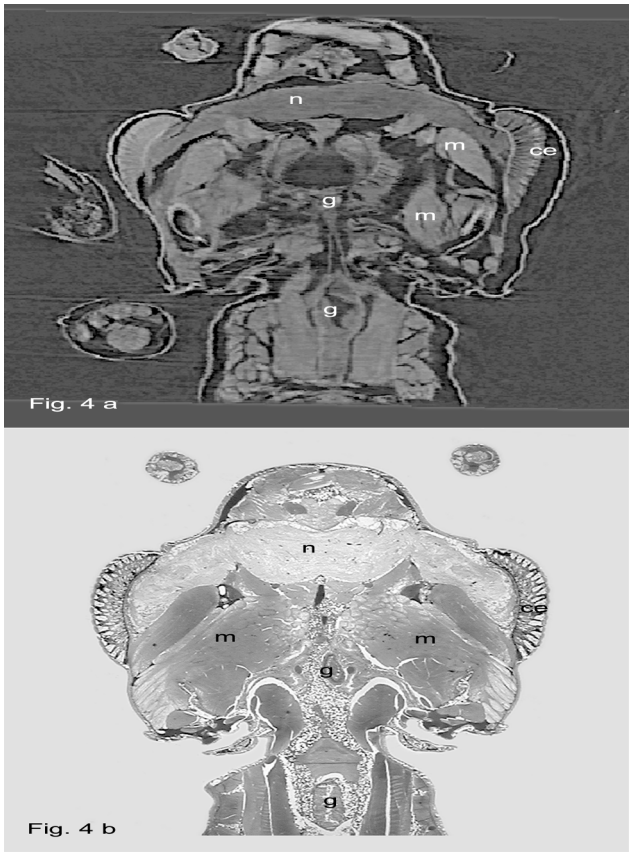

FIG. 4. Scutigera coleoptrata. (a) Tomographic data. (b) Histological section. g = gut, n = nerve tissue, m = musculature, ce = complex-eyes.

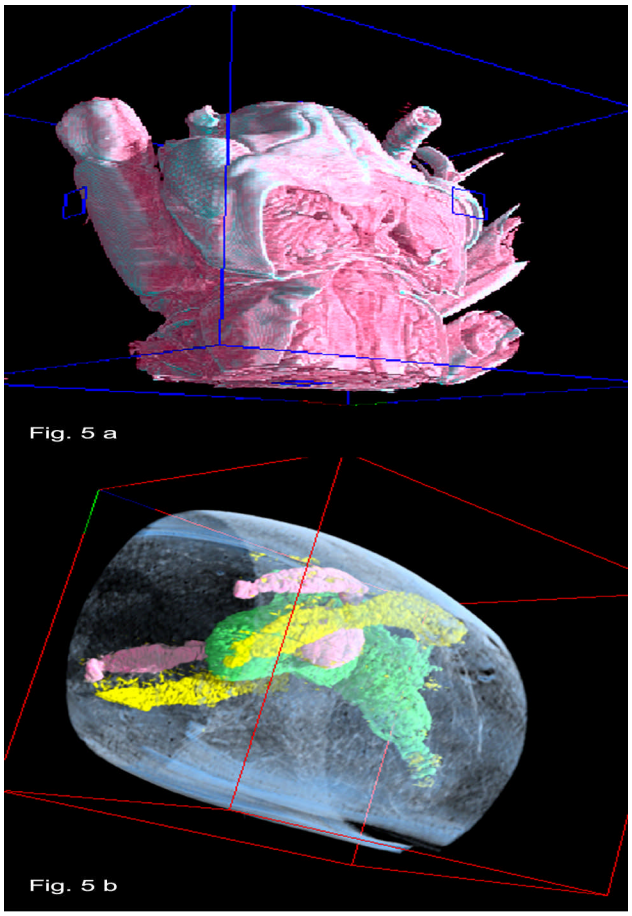

FIG. 5. Visualizations of the Scutigera data. (a) Cuticle (cyan), soft tissues (red), and the ostracod. (b) Gut (green), midgut-gland (yellow), ovaria (pink), and shell (blue).

also be minimized in the future. Therefore, hundreds of specimens could be scanned in typical beamline periods of 48-72 h. A comparable result — a series of digitized section images — typically takes at least 1 wk when histological techniques are used. The procedure would be as follows: resin embedding for 3 d, microtome sectioning and staining for 1 d, data acquisition with a microscope and CCD camera for 1 d, and data processing and alignment with suitable graphic software for 2 d.

The most serious problem is the low contrast of the data. Since the data show nearly exceptional absorption contrast, the problem can be easily understood: In the range of energy mentioned (7.5 keV), it is mostly the carbon that absorbs radiation. Therefore, the different kinds of tissue will show nearly the same grey-scale range, since the carbon content is almost identical. A routine applicability of x-ray microtomography in invertebrate micro-anatomical research will depend on techniques that will allow tissue-specific staining (e.g., heavy metals or heavy-metal-coupled antibodies) in order to enhance the contrast of certain tissues. Another promising account might be the acquisition of a phase contrast signal [3], which, in principle, should be possible, since x-ray undulator radiation shows a high degree of spatial coherence [4].

Acknowledgments

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