

Submitting author: Martin Fanenbruck; e-mail: martin.fanenbruck@ruhr-uni-bochum.de

co-authors: Francesco De Carlo: decarlo@aps.anl.gov

Derrick C. Mancini: mancini@aps.anl.gov

Report I.D.: fanenbruckm1.doc

fanenbruckm1F1.tif

fanenbruckm1F2.tif

fanenbruckm1F3.tif

fanenbruckm1F4.tif

fanenbruckm1F5.tif

Conducted at: SRI-CAT

Scientific Discipline: Biological Science

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

M. Fanenbruck,¹ F. De Carlo,² D. C. Mancini,²

¹ Lehrstuhl für Spezielle Zoologie, Ruhr.Universität Bochum, Germany

² Argonne National Laboratory, Argonne, IL, U.S.A

Introduction

Anatomical research in biological samples in a range of size of a few mm are usually extremely time consuming. Such studies typically are done by the analysis of histological section series. Mechanical artefacts caused by the cutting process mostly hinder the automatization of the 3d-reconstruction. Computer aided reconstructions of histological data may produce excellent visualizations [1] but the time effort is some months per specimen. X-ray micro-tomography seems to be a promising alternative, since mechanical artefacts could be completely avoided. The data-acquisition time recently has become rather short due to the high brilliance of radiation provided by third generation synchrotron sources like the Advanced Photon Source (APS) [2].

The goal of the recent study was to evaluate the data quality of x-ray microtomography compared to histological data and its advantage for the field of work mentioned above.

Methods and Materials

A straight-ray projection microtomographic system as described in [2] was used at the APS 2BM-beamline. The x-ray energy was varied from 6.0 to 12.0 keV using a multilayer monochromator. A CdWO₄ (10.0 x 10.0 x 0.5 mm) scintillator screen was used. A set of Zeiss AXIOPLAN (5x, 10x, 20x) microscopic objective lenses was used. The CCD-camera was a peltier cooled MicroImager II, QImaging. 720 projections of 1024 x 1024 pixel were taken rotating the samples in increments of 0.25° around 180° by a microstep rotary stage. The acquisition time was 0.5 sec per projection.

Black field (x-ray beamshutter closed) and white field (beam without sample) were taken in steps of 20 projections. The Data size was reduced to 512 x 512 pixel per projection after

normalization. A set of 512 tomographic sections was reconstructed by a filtered back-projection algorithm using the massive parallel linux-cluster available in Sector 2 of the APS. 3D-visualization and segmentation was done using the PC software package VGStudioMax, Volume Graphics.

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

Results

A 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.



Fig.1: Normalized projection of an ostracod crustacean specimen (b) of approx. 0,8 mm total length.

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

The maximum resolution can be estimated in the ostracod crustacean, using a 20x-objective (Fig. 3). Some massive muscle bundles obviously show myo-striation (Z-band distance approx. 2.5 μm). Therefore the resolution achieved here was around 1 micron.

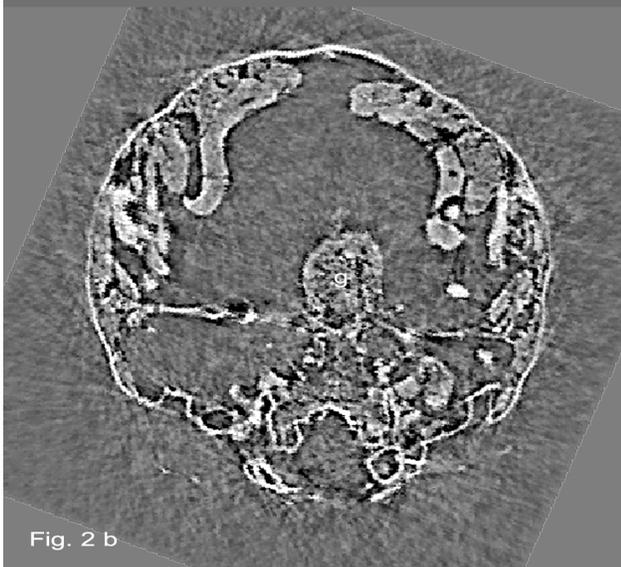


Fig. 2: Tomographic sections of the copepod crustacean specimen *Cyclops sp.*, (sample length 1mm) scanned in wet (a) and dried condition (b).

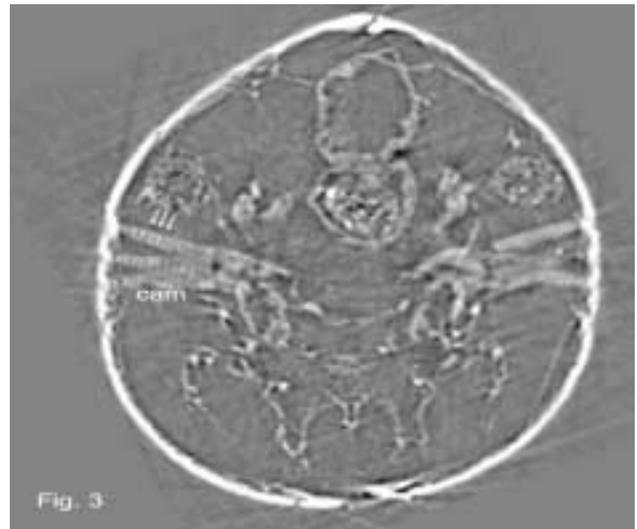


Fig. 3: Ostracod specimen, showing muscular striations in carapace adductor muscle (cam).

Comparing the tomographic data quality to histological sections we notice that almost all tissues shown by histology are clearly discernible in the tomographic data (Fig. 4 a, b) too.

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

Discussion

Since the acquisition time was 0.5 sec. per projection, the whole data set principally might be acquired in less than 10 min. It was about (solvable) technical problems why the total acquisition time was around one hour in practise. Additionally 15 min. are needed for data processing that might be minimized too in future. Therefore hundreds of specimen could be scanned in typical beam-line periods of 48 - 72 hours. A comparable result – a series of digitised section images – typically takes at least one week using histological techniques. The procedure would be as follows: resin embedding (3 days), microtome sectioning and staining (1 day), data acquisition using a microscope and a CCD-camera (1 day), data processing and alignment with suitable graphic software (2 days).

Nevertheless 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 mentioned range of energy (7.5 keV) it is mostly the carbon, which absorbs radiation. Therefore the different kinds of tissue will show nearly the same grey-scale range, as the content of carbon is almost identical. A routine applicability of x-ray microtomography in invertebrate micro-anatomical research will depend on techniques, which will allow a 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 principally should be possible, since x-ray undulator radiation shows a high degree of spatial coherence [4].

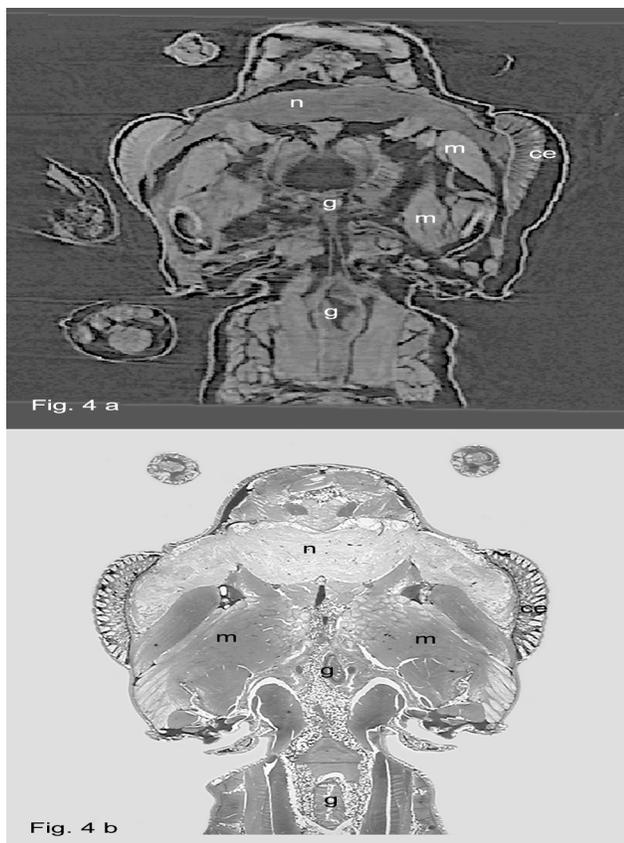


Fig. 4: *Scutigera coleoptrata*: (a) tomographic data, (b) histological section, g: gut, n: nervous tissue; m: musculature, ce: complex-eyes.

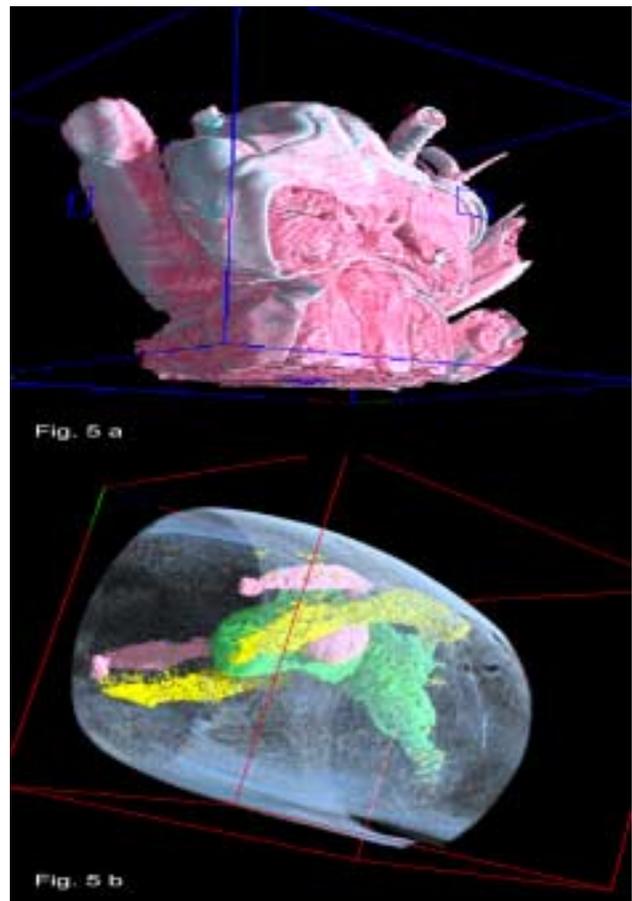


Fig. 5: Visualizations of the *Scutigera* data (a):cuticle (cyan), soft tissues (red) and the ostracod (b): gut (green), midgut-gland (yellow), ovaria (pink), shell (blue).

Acknowledgements

This work was granted by the German DFG, Project No. WA 530/23-2. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, under Contract No. W-31-109-ENG-38.

References

- [1] R. G. Beutel, A. Haas, *Zoomorphology* 18, 103-116 (1998)
- [2] Y. Wang et al., *Rev. Sci. Instrum.* 4, 2062-2068 (2001)
- [3] A. Barty, K. A. Nugent, A. Roberts, D. Paganin, *Opt. Commun.* 175, 329-336 (2000)
- [4] D. Paterson et al., *Opt. Commun.* 195, 79-84 (2001)