Visualization of movements of ventilatory muscles in the prothorax and 3-D reconstruction of the respiratory system of flies on the basis of x-ray radiography and micro-tomography

L. T. Wasserthal[1), A. Rack[2)

[1) University of Erlangen, Institute of Biology, Staudstrasse, D-91058 Erlangen, Germany
[2) Forschungszentrum Karlsruhe GmbH, Institute for Synchrotron Radiation, Hermann-von-Helmholtz-Platz 1, D-76344 Eggenstein-Leopoldshafen, Germany

While x-ray-tomography and radio-videography are established techniques in anatomical analyses of larger animals and humans the technique to use x-rays for analysis of small animals such as insects became successful in the last years with the optimization of the Synchrotron facilities.
Application of coherent Synchrotron x-ray beams with high density enables to obtain sufficient contrast and spatial resolution even in weakly absorbing insects which are void of mineralized skeletons in contrast to vertebrates. In video-radiography, the high intensity is achieved at the cost of irreversible damaging the living insect. In the 3-D volume reconstructions on the basis of micro-tomographs of fixed organisms, the danger arises to produce artifacts during long exposure times. Despite these disadvantages, the application of x-rays in structural analyses is a promising method, which allows to supplement physiological measurements and structural reconstructions of the mechanically intact objects. After a successful work with monochromatic beam of the ESRF at the beamline ID19 [1], it was interesting to get experience with the white beam of the TOPO-TOMO beamline at ANKA using similar objects and procedures.

1) X-ray video radiography of the anterior body of the blowfly Calliphora vicina.

In x-ray videos from the ESRF we had already observed pulsations in the posterior head and anterior thorax which were produced by muscles active only during forward pulse periods of the heart. As we had only videos from the lateral view, the precise extension of these muscles and the number of muscles involved in these pulsations remained unclear. Therefore we made videos from the flies in the dorso-ventral orientation at the ANKA TopoTomo beamline. It was possible to make videos with 100 to 500 frames at a frequency of 50 Hz with a total field of view of 5.5 mm x 5.5 mm. A 1 mm silicon absoption filter was used in order to suppress the soft part of TopoTomo‘s spectrum. As observed already at the ESRF beamline, the first sequences were disturbed by erratic movements of all muscle systems, e.g. the mouthparts, the head and legs. After about 4 to 6 s irradiation of the anterior body, the erratic muscle activity was reduced or blocked and only the myogenic systems like intestine, heart and accessory pulsatile organs remained active. After 24 – 30 s exposure time the pulse acitivity also of these muscular organs fully stopped. Therefore only video sequences after 8 s and before 20 s total irradiation time allowed analyses of the interesting muscle systems.
As expected, at least the cephalic pulsatile organ (CPO) and the longest pleuro-cervical muscle (PCM) are involved in the recorded contractions (Fig. 1). Probably two further pleuro-cervical muscles, which all converge and insert inside the neck, contribute to the complex movement of the long PCM muscle. As physiological pressure measurements revealed, these muscle contractions are involved in hemolymph transpoprt and air ventilation in the fly.
Comparison of the videos from ANKA with the

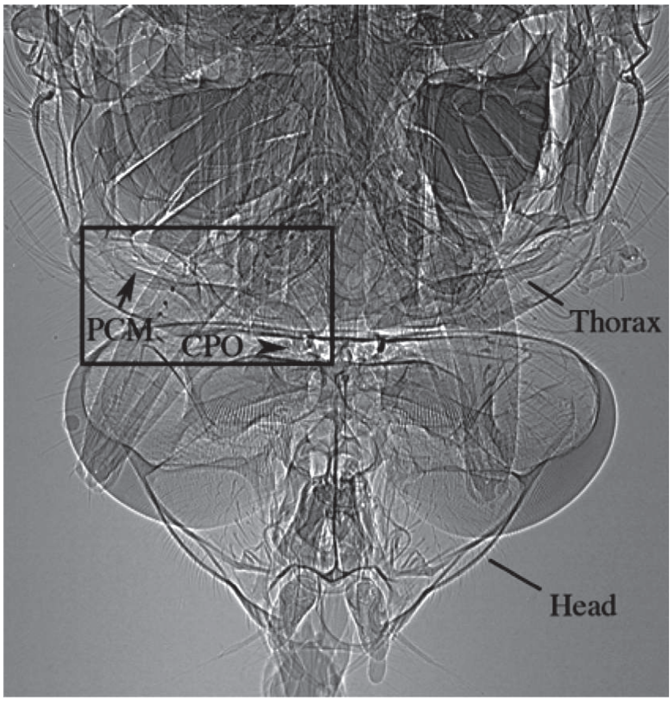

Figure 1: *X-ray frame from a video radiograph of the anterior body of the blowfly Calliphora vicina. (Cal2 s3) The position of the cephalic pulsatile organ (CPO) and the long prothoracic pleurocervical muscle (PCM) are indicatet by arrows inside the boxed region.*

ESRF clearly showed that the spatial resolution and contrast at ANKA was as well or better as at the ESRF despite or due to the higher frame rate (50Hz compared to 19 Hz). However the white x-ray beam is much more destructive than the monochromatic beam applied with similar parameters. Flies at the ESRF-beamline ID19 survived several hours and the myogenic pulsations of the accessory pulsatile organs and heart allowed 43 sequences with 100 to 250 frames within 3 – 4 hours and a sum of up to 7 minutes versus 20 s at ANKA of exposition before they stopped.

2) 3-D reconstruction of the abdominal tracheal system in flies on the basis of micro computer tomographs.

Already during the running expositions it could be observed that movements of the contents inside the gut of chemically fixed flies have prevented to obtain a stable stack of frames with continuous contours. Obviously there are x-ray-induced processes which enlarge the diameter and number of bubbles inside the intestine at the expense of the volume of the abdominal air sacs of the respiratory system, which got reduced from the original 2/3 to 1/3 of the abdominal volume within about an hour exposure time. Therefore we renounced to perform the planned tomographs of the abdomen of Drosophila and Calliphora. Instead we scanned a fixed hover fly Eristalis which presumably had no fluids inside the intestine. These scans are, however, not yet fully processed.

Reference

[1] Wasserthal, L.T., Cloetens, P. and Fink, R. (2006). Synchrotron x-ray-videography and tomography combined with physiological measurements for analysis of circulation and respiration dynamics in insects (Drosophila and Calliphora). Deutsche Tagung für Forschung mit Synchrotronstrahlung, Neutronen und Ionenstrahlen an Großgeräten, Hamburg, F-V55.