Application of X-ray imaging techniques for studying the morphology of malaria mosquitoes

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Introduction

The purpose of the duty travel was to perform X-ray phase-contrast tomography of malaria transmitting mosquitoes (genus Anopheles) selected from irradiated and control groups as well as radiographic imaging of living and chemically fixed Tsetse flies (Glossina pallidipes). The X-ray phase-contrast imaging technique allows radiographic-like imaging of samples characterized by very weak or no absorption contrast. This technique is of special interest for radiography of soft tissue, small animals, and insects where there is not enough material to produce satisfactory absorption contrast. Another advantage of the technique over the X-ray absorption radiography is minimization of the radiation dose to the sample [1]. The technique enables investigating living animal species or performing tomographic imaging with longer exposition times minimizing radiation damage to the otherwise sensitive and delicate samples. The images of the malaria transmitting mosquitoes provide information about morphological differences between the irradiated and control groups of specimens, if any. It is of prime importance for a successful application of the sterile insect technique (SIT) that the irradiated (sterile) group of male mosquitoes is otherwise healthy and can compete with the local population of males during the mating. In case of the Tsetse flies there is also an interest in the in vivo investigation of mating process as well as in learning more about the morphology of specific organs, such as salivary glands and reproductive organs. The experiments were carried out in collaboration with the Entomology Unit, the Atominstitut, Vienna and the ANKA light source (Topo-Tomo BL) in Karlsruhe, Germany.

Experimental

Two measurement set ups were utilized. Set up #1 was equipped with a high magnification X-ray microscope consisting of single crystal scintilator, magnifying optics coupled to high-resolution, cooled CCD camera. The set up #1 image resolution was equal to about 2.5 μm (0.9 μm pixel size) and the field of view was about 1 mm x 1 mm. Set up #2 consisted of a medium magnification X-ray microscope with high brilliance optics/single crystal scintillator coupled to an ultrafast, cooled CMOS camera. The image resolution of the set up #2 was equal to about 20 μm (6 μm pixel size) and the field of view was equal to about 10 mm x 10 mm.

Two groups of samples were measured: (1) irradiated and control group mosquitoes: fixed abdomens and heads; (2) live mating Tsetse flies and fixed Tsetse flies. The chemically fixed mosquito and Tsetse fly samples were measured in computed tomography (CT) mode in the set up #1. The in vivo mating of Tsetse flies was captured by using the set up #2. In Fig. 1 the picture of the Topo-Tomo beam line and the set up #2 is shown.

During the experiment X-ray phase contrast enhanced images were collected. These data are being evaluated. Two examples of the collected data

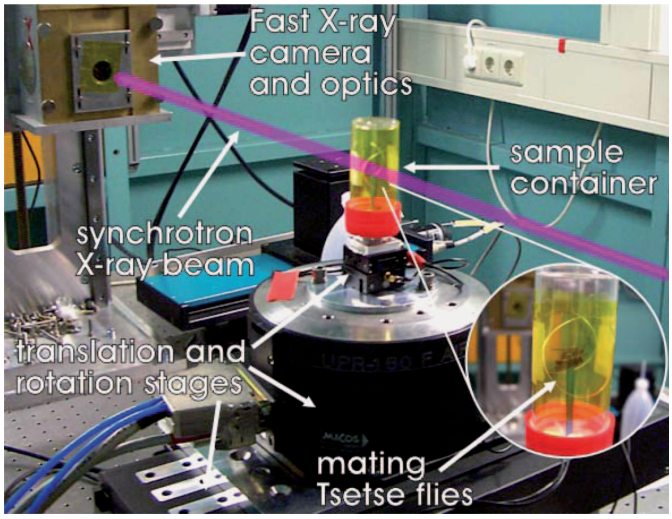

Figure 1: *Picture of the set up no. 2 at the Tomo-Topo beam line. The set up no. 2 comprised a fast X-ray camera/optics capable of taking phase-contrast image sequences at 125 frames per second. A close-up view of the sample container with mating Tsetse flies is also shown.*

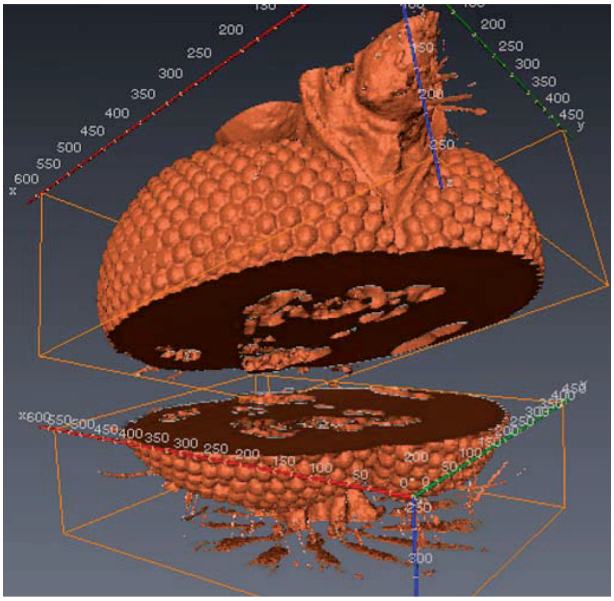

***Figure 2**: Volumetric reconstruction of mosquito head (control population). A virtual cross-section through the sample shows the internal void regions. The CT scan consisted of 1000 projections, the distances in the image are given in micrometers.*

sets are given in Figs. 2 and 3. In Fig. 2 a volumetric reconstruction of a mosquito head is presented. This data set comes from CT measurement of a chemically fixed sample. The surface and internal morphological details can be inspected. However the grey level detail allows for differentiation between the voids and the fixed tissue only. A dedicated computer code has been developed to extract the projected sample thickness from X-ray phase contrast images, based on a approach proposed in [2].

The imaging of live species of Tsetse flies was performed inside specially designed plastic containers. In the container a single female species was attached with a glue to a pole and the male was let free. The container was closed. The X-ray beam passed through two windows (entrance and exit) made of 7.5 μm thick Kapton foil. During the mating a sequence of phase-contrast images was taken with a speed 125 frames per second. A few frames from such a sequence are shown in Fig. 3.

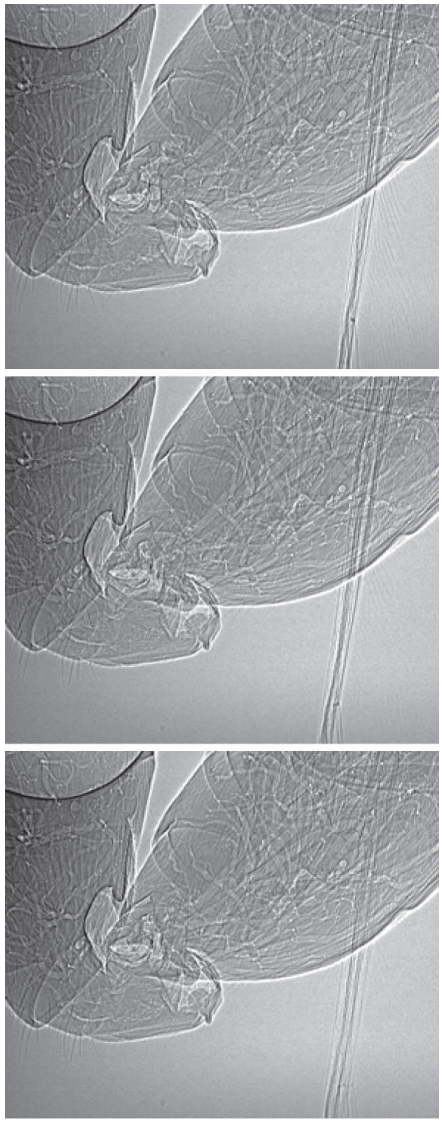

***Figure 3:** Three frames (40 ms time distance) from a of sequence 14687 images taken by a fast X-ray micro-imaging (set up #2) during mating of Tsetse flies.*

Conclusions

The elaborated preliminary data were examined. The entomologists have confirmed that there is a great interest in imaging of morphological details of mosquito and fly species. Especially very unique data are provided by imaging of live species, and *in vivo* processes. This type of X-ray imaging of weakly absorbing samples has been made possible very recently and it is of great interest for life science research. Regarding the CT imaging of mosquito species the material collected so far has to be still examined with collaboration of the entomologists. The general conclusion is that some of the internal morphological details of mosquito organs can be extracted from the collected CT data, especially when the organs are separated by voids. However there is a need to modify or develop new sample fixing procedures to improve X-ray phase contrast for specific organs and structures within the mosquito body as part of a needed future collaboration between physicists and entomologists.

References

[1] J.J. Socha, M.W. Westneat, J.F. Harrison, J.S. Waters, W.K. Lee, Real-time phase-contrast X-ray imaging: a new technique for the study of animal form and function, BMC Biology 5:6, (2007), http://www.biomedcentral.com//1741 – 7007/5/6.

[2] D. Paganin, S.C. Mayo, T.E. Gureyev, P.R. Miller, S.W. Wilkins, Simultaneous phase and amplitude extraction from a single defocused image of a homogeneous object, Journal of Microscopy 2006, 33-40 (2002)