

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

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Introduction

X-ray phase contrast tomography technique was applied to examine the morphology of malaria transmitting mosquitoes in support to the development of the sterile insect technique (SIT) [1, 2]. The aim of the experiment was to detect eventual damage induced by the sample preparation procedures, to perform X-ray phase-contrast imaging on freshly prepared (not fixed) and live mosquito species, and to test the new beamline set up, which was not yet fully commissioned at the time of the experiment.

Experimental

During current experiment, which was performed in the TOPO beamline, a new cooled and thermally stabilized CCD camera PCO4000 was used. The camera was coupled to a thin layer scintillator screen and optics. A white beam from a bending magnet was used for the in-line X-ray phase-contrast imaging. The details of the set up are shown in Fig. 1.

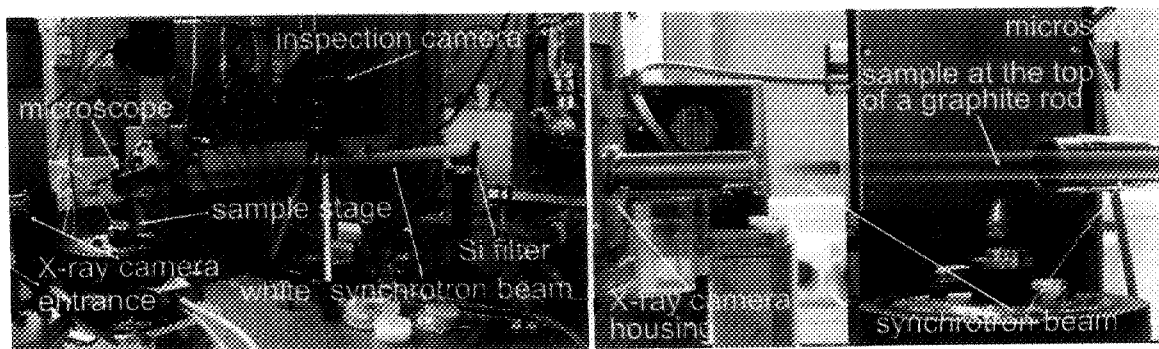


Fig. 1. X-ray phase contrast imaging set up at the TOPO beamline, ANKA Synchrotron Facility. Left picture shows overall view of the set up, in the right-hand side picture a close up view of the "sample - X-ray camera" region is shown.

The spatial resolution of the set up was about 2.5 micrometers per image pixel. Relatively poor spatial resolution did not allow imaging the morphological details at the tissue level. However, due to a large field of view, it allowed for imaging whole live mosquito species using relative short exposition times (200-400 milliseconds per image).

Selected mosquito species were fixed using procedures developed in the Instrumentation Unit (IAEA). The live specimens were hatch from larvae mosquito forms. The larvae culture was prepared in the Entomology Unit (IAEA) two days before departure to ANKA. The hatching of adult mosquitoes took place in the ANKA chemistry laboratory in an isolated hatching cage shown in Fig. 2. A series of X-ray phase contrast measurements was carried out. The collected data included still images of fixed male mosquitoes representing irradiated/control populations (14/14 abdomens, 15/14 heads), CT scans of whole mosquitoes (3/3), abdomens (5/5), and heads (4/5). Also sequences of phase contrast images (movies) of living specimens (4 male, 3 female, 3 pupae, and one larva) were collected. The CT scans were also performed for not fixed, freshly prepared, female mosquitoes (2). Still images of not fixed male (1) and females (3) were also taken, as well as CT scans of fixed fruit flies (6). Altogether about 208 GB data was collected. In Fig. 3 a few frames from the collected image sequences of living specimens are shown.

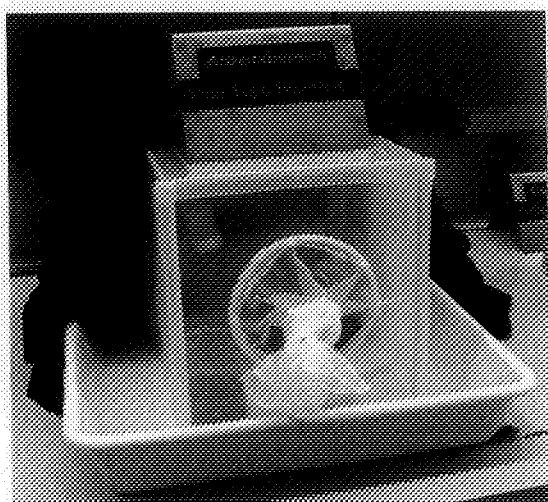


Fig. 2. Mosquito hatching cage installed in the ANKA chemistry laboratory – the source of live and not fixed mosquito specimens.

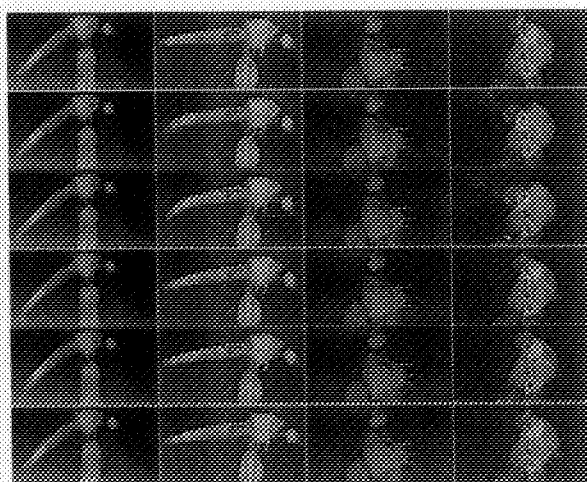


Fig. 3. Individual frames taken out from four X-ray phase contrast image sequences showing internal morphology of living mosquito specimens. From left to right: two female and two male mosquitoes, frame rate of 4, 2.5, 5, and 4 frames per second, respectively.

The frame rate was in the range between 2.5 – 4 frames per second. It was the first experiment in which the interactions between the internal organs of a mosquito were observed. The collected data showed also damage induced to the sample due to the fixation procedure. This is demonstrated in Fig. 4. The cracks visible in the reconstructed cross-section of a fixed mosquito head are due to too fast dehydration of the sample. In this picture there are also some artefacts visible. These artefacts could arise from the sample motion during the CT scan or the misalignment/instability of the scanning stage.

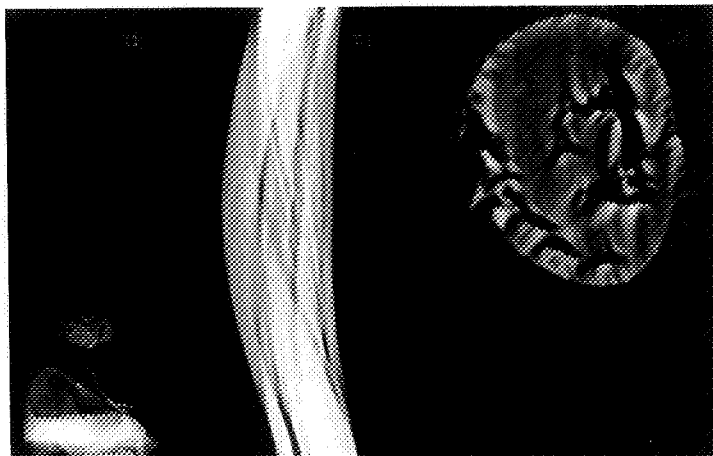


Fig. 4. Left to right: (a) single projection image (phase retrieved) from a series of 1001 projections of a fixed male mosquito head; (b) sinogram build from the 1001 projections at the height of the mosquito head (1150th line in the image shown in (a)); (c) reconstructed cross-section through the mosquito head – notice the cracks shown as black regions in the reconstructed cross-section).

Conclusions

The ability to perform X-ray phase-contrast imaging of live mosquito specimens was confirmed. The collected still images provided data on a relatively large population of mosquitoes. The CT data were very useful to compare selected mosquito species. They confirmed that the sample preparation procedures are critical for examining the morphological details. The procedures must be further optimized in order to stabilize the sample without inducing significant damage. The most interesting results should be obtained with the high-resolution (~ 0.5 micrometer) set up using the FReloN camera to be commissioned at the TOPO beamline in the 3rd quarter of 2007. If there are differences between the control and irradiated populations of mosquitoes they should show up first at the tissue level. Using the high-resolution set up it should be possible to detect such differences, if present.

References

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