ESRF	**Experiment title:**	**Experiment number**: SC 1347
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Shifts: 6	**Local contact(s)**: Peter Cloetens	*Received at ESRF:*

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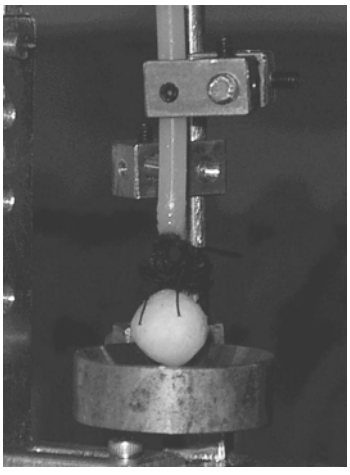
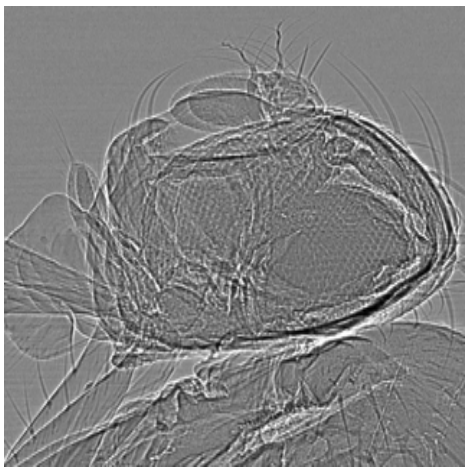
Report:

As announced in our proposal we recorded the movements inside the heads of living insects:
1. the blowfly (Calliphora vicina),
2. the hoverfly (Eristalis tenax) and
3. the fruitfly (Drosophila 6).

In the blowflies and the hoverflies we successfully recorded in parallel the intratracheal pressure during x-ray exposure.

The living flies were mounted in a holder with a direct connection with the pressure sensor to the dorsal air sac of the respiratory apparatus either in a vertical or in a horizontal position. To calm the insect it was allowed to hold a ball of styrofoam with the legs during the experiments (see below, left image). The air pressure data were continuously recorded on a Apple Mac Powerbook using a custom made amplifier and a Mac Lab AD-interface. The recording of the rhythmic pulsatory activity was initiated several minutes before exposure to the beam. In the video mode the 2D detector made 20 frames per second with a 2 x 2 mm² or 1 x 1 mm² field of view (see below for drosophila) and 512 x 512 pixels resolution. Exposure time of each frame was 1 to 10 ms and the video sequences lasted between 200 and 3000 frames. Every recorded image gave a trigger signal and thus could directly be correlated with the tracheal movements.

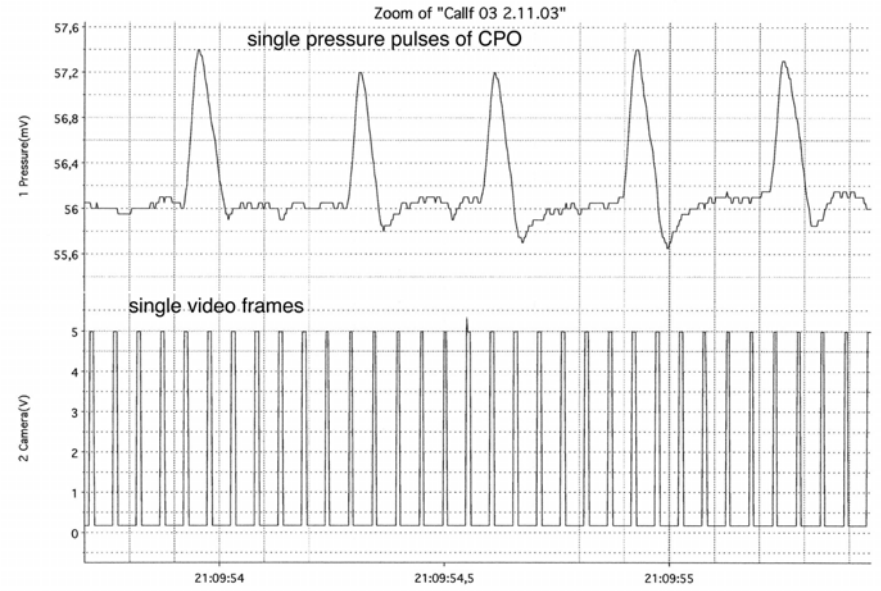




The influence of the x-ray beam upon the respiratory pulsations in the head was obvious. It resulted in a drastic increase of pulse rate and change of pulse amplitude. Correspondingly the periods of the intermittent pulse activity became shorter as it would happen after an increase in temperature. After stopping the x-ray exposition, these flies recovered slowly and regained the former resting rhythm.

From the video sequences in combination with the detection of the intratracheal pressure it can clearly be shown that the single pressure pulses coincide with the pulsatory activity of the cephalic pulsatile organ (CPO) which is responsible for the ventilatory movements of the cephalic air sacs.

The survival time of the insects was very different: While the experiments in the larger flies could be conducted over several minutes and with intervening long pauses for recovery even up to two hours, the muscles in the heads of the smaller Drosophila were immobilized already after an exposition to 3x1000 frames. But it was possible also in these delicate insects for the first time to visualize the activity of the cephalic pulsatile organ and the periodic volume change of the air sacs in the head and to scan a head of a dead specimen for 3-D tomography.



Recordings of intratracheal pressure in the blowfly Calliphora and parallel trigger signals of video camera at ESRF ID 19

Thus, we could demonstrate that - inspite of the drastic effects of the x-rays upon the living insect - insights into the functioning of the internal structures are possible and thus the method ideally contributes to the understanding of the physiological processes.