Atomic force microscopy to detect internal live processes in insects

M. E. Dokukin,1 N. V. Guz,1 S. Vasilyev,1 and I. Sokolov1,2,a)

1Department of Physics, Clarkson University, 8 Clarkson Ave., New York 13699, USA
2Nanoengineering and Biotechnology Laboratories Center (NABLAB), 8 Clarkson Ave., Clarkson University, New York 13699, USA

(Received 10 November 2009; accepted 17 November 2009; published online 27 January 2010)

Here we report on the use of atomic force microscopy (AFM) to study surface oscillations coming from internal live processes of insects. With a specially designed AFM stage to keep an insect motion partially restricted, the AFM can record internal oscillations on different parts of the insect. We demonstrate the method for a fly, mosquito, and lady beetle. We show that AFM can provide information about the spectral behavior that has not been studied so far, 10–600 Hz range, detecting amplitudes down to subnanometer level. © 2010 American Institute of Physics.

Insects are the most numerous and diverse group of animals. They are also highly efficient bio-machines varying greatly in size. Studies in insect physiology continue to reveal unknown mechanisms in respiration,1,2 communication,3 and other aspects of insect functioning.4 While physiology/biophysics of insects is quite an active area of research,5 little exploration has been done with modern nanotechnology tools.

The atomic force microscopy (AFM) technique has become popular in the study of biological materials.6–12 AFM is capable of measuring motion of the surface of biological cells at the level of several nanometers.6,8,11–15 Expansion of this technique to larger living objects, like insects, has been restricted by the detection range of vertical motion of the AFM probe (typically ~50 μm). Organ and body movements in a living insect easily exceed this range. To protect the integrity of the AFM cantilever and to use AFM to record motion of an insect surface, one needs to restrict the motion of the insect. In order to restrict the motion of a living insect, we built a special stage, Fig. 1(a). The key element of the stage is a thin metallic membrane with an opening of a few millimeters in diameter. Specific diameter depends on the type of the insect of interest. In this work, we used 2 and 5 mm openings. The insect was attached from underneath of the membrane, Fig. 1(a), with the help of either an adhesive tape or a microscopic amount of glue (the latter attachment is sacrificial for the insect). This minimizes the insect motion in vertical direction and virtually excludes any movement in lateral directions. In order to restrict lateral motions of the insect in the case of adhesive tape, a double sticky tape was attached around the aperture of the membrane. This allows restricting lateral motion while keeping insects relatively weakly squeezed with the adhesive tape (which might otherwise be damaging for the insect).

An AFM probe was positioned on the top of the insect through the aperture in the holder membrane with the help of built-in optical microscope, Fig. 1(a). The scan size was set to 0 nm and scan rates were chosen from 0.1 to 1 Hz. To prevent a possible influence of the feedback, the scan feedback gain parameters were set between 0 to 0.01 for the integral, and to zero for the proportional gain. The spectral signals shown in this paper were collected when both integral and proportional gains were set to 0.

All vibrations of the insect surfaces were recorded by means of a Veeco Dimension 3100 AFM equipped with NanoScope V controller (all other versions of the controller could also be used). Veeco silicon nitride integrate DNP probes with spring constant of the cantilever k ~ 0.06 N/m were used in this study. The signal, vertical position of the AFM cantilever was recorded by direct sampling using a National Instruments ADC 24-bit card (NI PCI-5922) card at 50 kHz. Simultaneously, the external sound was recorded by means of a wideband microphone. The sound was sampled using the second input channel of the same ADC card. LABVIEW (by National Instruments) version 8.2 was used as the data collection interface. A schematic of the experiments is shown in Fig. 1(b). The AFM probe can be located on the surface of the attached insect with nanometer precision by using the AFM piezo scanner, while roughly positioned with a built-in optical system.

Here we recorded the change in vertical position of the insect body surface of mosquitoes (Anopheles), flies (Musca domestica), and lady bird beetles (Hippodamia convergens). Typical signals collected from the elytra of a lady beetle are shown in Fig. 1(a). The AFM probe can be located on the surface of the attached insect with nanometer precision by using the AFM piezo scanner, while roughly positioned with a built-in optical system.

We demonstrate the method for a fly, mosquito, and lady beetle. We show that AFM can provide information about the spectral behavior that has not been studied so far, 10–600 Hz range, detecting amplitudes down to subnanometer level. © 2010 American Institute of Physics.

FIG. 1. (Color online) A scheme of the experimental setup. (a) The special insect holder to restrict motion of the insect during the AFM study. (b) An AFM probe put in contact with an insect surface at an optically chosen place, and the deflection signal from of the AFM cantilever was recorded. Data from a microphone placed near the insect is used for the analysis of external noise.
shown in Fig. 2. One can see well-known periodic breathing, heartbeat cycles, and probably coelopulses at low frequencies [Fig. 2(a)]. The signals corresponding to the higher frequencies, shown in zoomed region of Fig. 2(a), have not been detected before presumably due to their small amplitude, which prevented them from detected above the noise level. Such signals are quite abundant, see Fig. 2(b), in which the low-frequency oscillations were removed with a low-pass filter. We found it interesting to translate the recorded oscillations into audible sound. Several audio files demonstrating the internal sound of mosquitoes, flies, and lady beetles can be found in Ref. 22.

To study the recorded signals quantitatively, one can analyze their Fourier spectra. As an example, we demonstrate the study of spectral behavior of lady beetle signals. Representative spectra are shown in Fig. 3(a). To exclude room noise, it was recorded with a wide-band microphone. To avoid confusion with the instrumental noise, the signal collected from a dead lady beetle, is also recorded as a reference. Substantial differences compared to the reference spectrum of the dead beetle are observed for the frequencies lesser than ~600 Hz. One can see substantial artifacts at 650, 720, and 800–1000 Hz. Comparing these frequencies with the sound spectrum recorded by microphone [Fig. 3(b)], we can conclude that all named artifacts are due to an intrinsic noise in the AFM instrument. Excluding these artifacts and room noise, one can identify the following frequencies typical for living lady beetles only: 47, 150, 187, 350, 480, 530, 590, and 600 Hz [shown with the arrows in Fig. 3(a)]. It is important to discuss the physical origin of the observed spectral peaks shown in Fig. 3(a). Intrinsic resonances of the AFM cantilever/holder can only be found above ~6 kHz, and consequently, do not contribute to the observed spectral peaks. The frequencies of the peaks are substantially higher than the frequencies of breathing, gut peristalsis or heart beating. Most probably, these signals are originated at the contraction of muscles of internal organs. The specific relation to particular organs will be studied in future works. Here, however, it is important to show that the found peaks have internal rather than surface origin. The later could come, for example, from vibrations of cuticular hairs and their sliding on wings due to breathing, heart beating, etc. To understand it further, we use a piezoactuator (close-loop 200×200×30 μm scanner by NPoint, Inc.) to mimic breathing/heart beating motion of a dead lady beetle by pushing its abdominal segments [Fig. 3(c) insert] with the frequency of 0.7 Hz (the low frequency of high amplitude oscillations shown in Fig. 2). The amplitude of the scanner was chosen to be ~10 microns to match the breathing amplitude measured on elytra (~300–500 nm). This presumably should produce mostly surface noise because of sliding wings, cuticular hairs, abdominal segments, elytra, etc. (we did not let the beetle dry). Comparing to the reference curves (1 and 3) of Fig. 3(c), one can see that the surface noise produced by the sliding parts of the dead beetle, Fig. 3 (curve 2), has two fairly broad and noisy maxima in the regions of 80–150 and 220–300 Hz. These peaks are robust; their frequency positions changes very little on different beetles, and remain constant with the change of pushing (“breathing”) amplitude. It is interesting to note that the shape of observed 80–150 Hz spectral maximum resembles approximately the shape of 110–150 Hz maximum area of the living beetle spectrum, Fig. 3(a). The second observed 220–300 Hz area is sufficiently broad and has supposedly small amplitude to be reliably identified in the spectrum of living beetle. Thus, we can conclude that the surface signals bring almost negligible contribution to the measured here spectra of living insects. There is presumably only one feature of the observed spectra, 110–150 Hz maximum area showed in Fig. 3, which has the surface (or sliding noise) origin.

To conclude, the described AFM-based technique is capable of detecting internal oscillations on the surface of living insects. Studying the spectra of recorded oscillations, one can learn mechanical/physiological processes inside the insect organism in a noninvasive manner.
ology” OR “insects AND physiology” OR “insects AND biophysics” OR “insect AND biophysics.”


23See supplementary material at http://dx.doi.org/10.1063/1.3273371; for the audio records of a lady beetle, fly and mosquito.