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Fluorescence-Based Intravital Imaging for the Study of Hemolymph Circulation in Mosquitoes

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Insects employ an open circulatory system for the transport of nutrients, wastes, and signaling molecules. The circulatory system also disseminates immune molecules and cells, promotes respiration, and is involved in thermoregulation. The general anatomy of the insect circulatory system is shared across taxa and consists of a dorsal vessel that extends the length of the insect, an open body cavity (hemocoel), and accessory pulsatile organs [1-3]. The primary hemolymph pumping organ, the dorsal vessel, is a muscular tube that runs along the dorsal midline of the insect and is divided into a thoracic aorta and an abdominal heart (Fig. 1). While a considerable amount of work has been directed toward characterizing the structure of the insect dorsal vessel [3], little is known about hemolymph flow in insects.

The present work describes a fluorescence-based intravital imaging method for the study of hemolymph circulation in mosquitoes. Here, mosquitoes are briefly cold-anesthetized and then restrained on Sylgard elastomer plates using non-invasive methods. After recovery from anesthesia, 1-2 μm fluorescent microspheres are intrathoracically injected into the mosquito hemocoel and allowed to mix with the hemolymph. Then, using a microscope connected to a low noise/high sensitivity camera, 60 sec videos are acquired under low-level fluorescence illumination and the general path of tracer particles monitored through time. Once major hemolymph flow lines are identified, the absolute flow of hemolymph is measured using particle-tracking software. Measurements obtained include distance traveled, distance from origin, velocity, velocity from origin, and acceleration (Fig. 1).

To date, we have used this procedure to show that (1) the mosquito heart propels hemolymph in both anterograde (toward the head) and retrograde (toward the tip of the abdomen) directions, (2) hemolymph enters the heart through paired valves (called ostia) located in each abdominal segment, (3) hemolymph exits the heart through distal excurrent openings (Fig. 2), (4) hemolymph flows fastest in the heart and slowest in specific regions of the abdomen, and (5) that ventral abdominal contractions potentiate retrograde extracardiac hemolymph propulsion during periods of anterograde heart flow [1,2]. Moreover, we are currently using this method to map absolute hemolymph currents in all areas of this insect.

In summary, we describe a fluorescence-based method for measuring hemolymph flow in insects. While other recent papers have described the use of bright field microscopy, synchrotron x-ray phase-contrast imaging, magnetic resonance imaging, and OCT imaging for the measurement of hemolymph flow (discussed in [4]), the protocol presented herein describes a high resolution, high sensitivity, and cost effective way of measuring hemolymph flow in insects.

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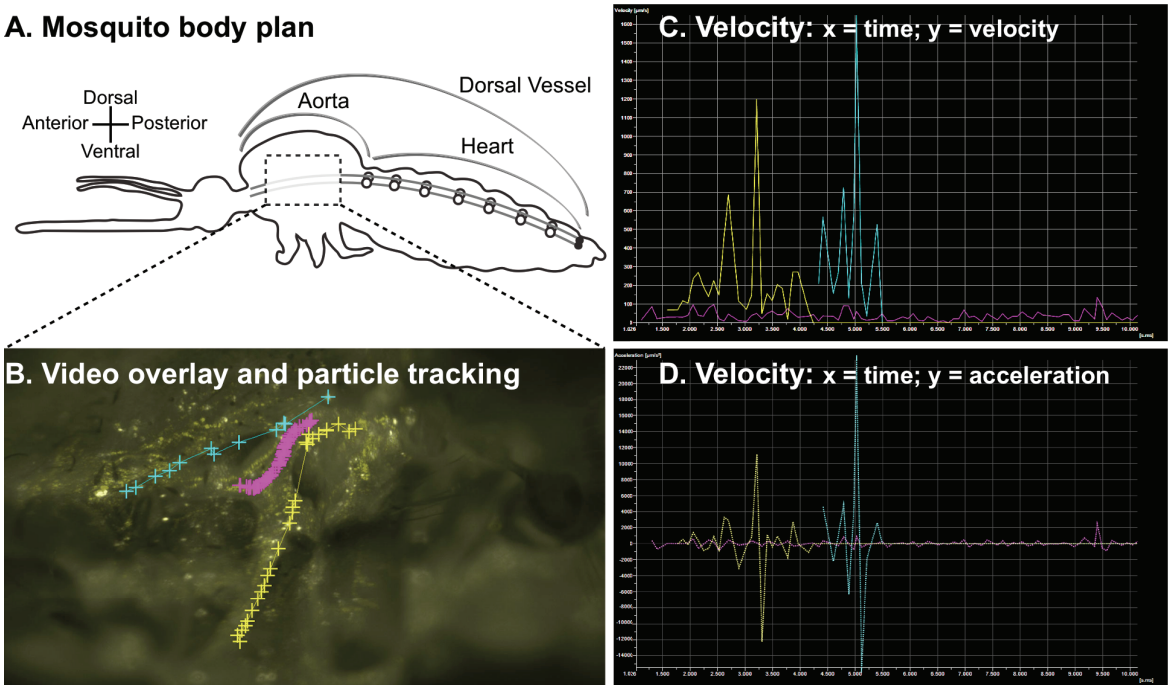

FIG. 1. Mosquito body plan and particle tracking in the hemocoel. A. Mosquito body plan showing the location of the dorsal vessel, which is divided into a thoracic aorta and an abdominal heart. B. Image overlay and particle tracking (colored lines) of a video showing fluorescent microspheres flowing through the lateral mesothorax (see dotted square in panel A). C-D. Velocity and acceleration of the three particles tracked in panel B.

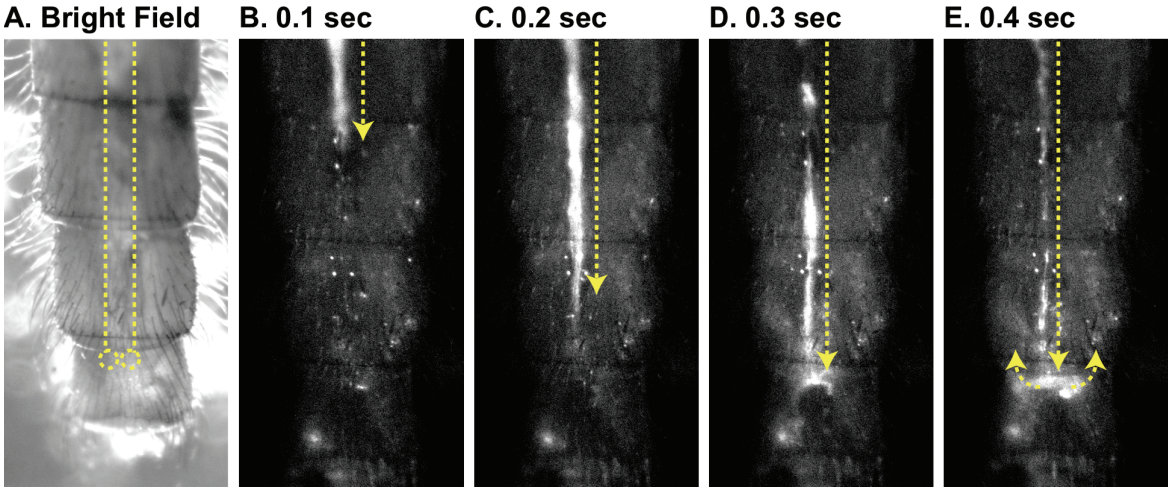

FIG. 2. Intravital imaging of the dorsal posterior abdomen, showing hemolymph exiting through the posterior excurrent opening. A. Bright field image of abdominal segments V-VIII. Parallel lines and circles delineate the position of the heart and the double-slit posterior excurrent opening, respectively. B-E. Time-lapse intravital imaging showing fluorescent particles being propelled through the heart in the retrograde direction and exiting through the excurrent opening. Modified from Glenn et al. [2].

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