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Protocol

Quantifying and Analyzing Mosquito Movement from Video Tracking Results

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After tracking the kinematics of a moving mosquito from video, one needs to convert the tracking output into the kinematics data needed to answer the research question. Here, we provide general guidelines for how to do this by discussing how to quantify body position and orientation in the world reference frame and wing and leg orientation in the mosquito body reference frame. These guidelines should be adapted based on the goal of your research. To answer your research question, the resulting kinematics data must then be further analyzed. Because the nature of this analysis depends strongly on your specific research question, we refer to literature for designing these postprocessing routines.

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MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

Equipment

Computer with Windows, Mac OS, or Linux operating system
Programming software such as Python, MATLAB, or R

METHOD

Here, we describe how to quantify the movement kinematics of mosquitoes from video tracking data. We first describe how to define a time–space reference frame for this. Then, we describe how to define the temporal dynamics of mosquito body position and orientation. Finally, we explain how to describe wing and leg kinematics.

Define the Coordinate System for Your Analysis

1. Define a world reference frame in two dimensions (2D) ($\mathbf{X} = \{x, y\}$) or three dimensions (3D) ($\mathbf{X} = \{x, y, z\}$) (see Fig. 1).

   The coordinate system of this world reference frame should be right-handed, and the coordinate axes should be explicitly defined. Position the center of the coordinate system in an appropriate location (e.g., the center of a landing platform), and orient the axes (e.g., set the y-axis or z-axis vertically up in 2D and 3D, respectively).

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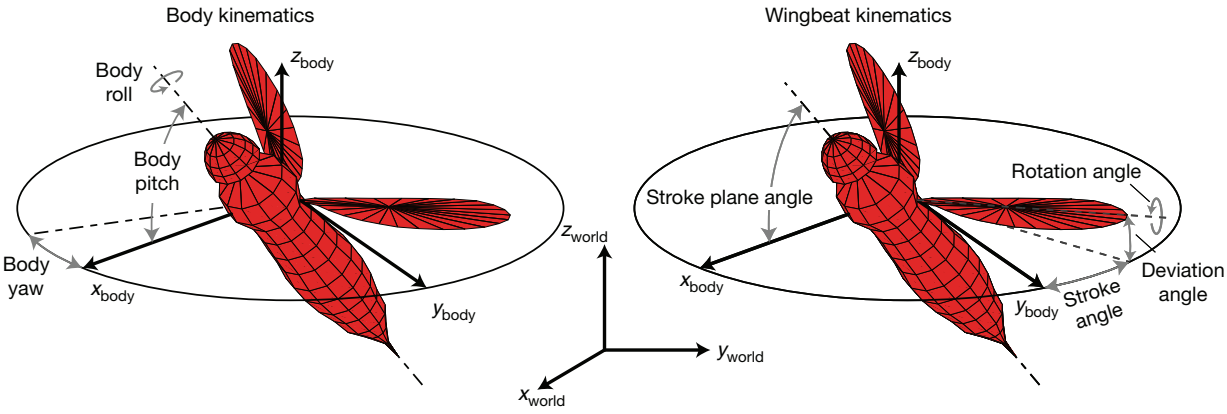

FIGURE 1. Definitions of the body and wingbeat kinematic parameters of flying mosquitoes. The *left* panel shows body orientation parameters: the three consecutive Euler angles body yaw, body pitch, and body roll. The *right* panel shows the wingbeat kinematics parameters: the three consecutive Euler angles wing stroke angle, wing deviation angle, and wing rotation angle. The body location and orientation are defined relative to the world reference frame with axes (x, y, z); The wingbeat kinematics angles are defined in the body-fixed stroke-plane reference frame, with a stroke plane angle of ~45° and with axes (x_{body}, y_{body}, z_{body}).

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2. Define time t, where $t = 0$ sec is a suitable moment in time (e.g., the start of the experiment or the moment of touchdown during a landing experiment).
3. Define all mosquito movement kinematics parameters within a video relative to this time–space coordinate system (t, $\mathbf{X}$).

Quantify the Position of a Moving Mosquito

For many studies, only the position of the moving mosquito is of relevance (e.g., Cribellier et al. 2020, 2022). Here, we explain how to define the temporal dynamics of this positional data throughout a video.

4. Define the position of a mosquito in each video frame as (t, $\mathbf{X}$).

   For a 2D single-camera study, this positional data will consist of the 2D array $\mathbf{X} = (x, y)$, where x is the horizontal position, and y is the equivalent vertical position. For 3D studies with multiple cameras, the positional data will consist of the 3D data $\mathbf{X} = (x, y, z)$.

5. Compile the positional data of all digitized video frames into a time–space array ($\mathbf{t}(n)$, $\mathbf{X}(n)$), where the video frame number n ranges from 1 to N from the first to the last digitized video frame.

   Here, the positional data thus consists of a set of two or three arrays for a 2D or 3D study, respectively: ($\mathbf{X} = (\mathbf{x}(n), \mathbf{y}(n))$ and $\mathbf{X} = (\mathbf{x}(n), \mathbf{y}(n), \mathbf{z}(n))$, respectively).

Quantify the Body Orientation of a Moving Mosquito

When tracking body orientation in addition to body location, these data need to be added to the kinematics time–space array using the following steps.

6. Define the orientation of the mosquito in each video frame using the body angle array $\mathbf{\Gamma}$.

   For 3D kinematics analyses, the body orientation array is defined as $\mathbf{\Gamma} = (\gamma, \beta, \theta)$, which consists of the three consecutive Euler angles, body yaw angle γ, body pitch angle β, and body roll angle θ (see Fig. 1).

   For 2D analyses with a single sideview camera, only body pitch β can be estimated, and thus the body angle array reduces to $\mathbf{\Gamma} = \beta$. Similarly, for studies with only a top view camera, only body yaw γ can be estimated, and thus the body angle array reduces to $\mathbf{\Gamma} = \gamma$.

7. Add the body kinematics array for all digitized video frames to the time–space kinematics array ($\mathbf{t}(n)$, $\mathbf{X}(n)$, $\mathbf{\Gamma}(n)$).

   Here, the body orientation angle array thus consists of three body angle arrays as $\mathbf{\Gamma}(n) = (\boldsymbol{\gamma}(n), \boldsymbol{\beta}(n), \boldsymbol{\theta}(n))$.

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Quantify the Wingbeat Kinematics of Flying Mosquitoes

When also tracking the wingbeat kinematics of a flying mosquito, the body kinematics time–space array should be augmented with a wing kinematics array, according to the following steps.

8. Define a wing stroke reference frame that is fixed to the mosquito body (see Fig. 1).

   This wing stroke reference frame should be located in a plane in which most wing stroke movements occur. For mosquitoes, this stroke plane is oriented at a stroke plane angle of ~45° relative to the long axis of the body (see Fig. 1; Muijres et al. 2017).

9. Define the orientation data of the left and right wing in this wing stroke reference frame using the wing angle arrays $\mathbf{\Phi}_L$, $\mathbf{\Phi}_R$, respectively. Each wing angle array $\mathbf{\Phi} = (\phi, \eta, \alpha)$ consists of the three consecutive wing kinematics Euler angles: the wing stroke angle ϕ, the wing deviation angle η, and the wing rotation angle α (see Fig. 1; Muijres et al. 2017).

   The stroke angle describes the wing rotation within the stroke plane; the deviation angle defines rotations out of the stroke plane; and the rotation angle defines wing pitch rotation around the wingspan axis.

10. Add the wing kinematics array of both wings to the complete time–space kinematics array for the digitized video $(\mathbf{t}(n), \mathbf{X}(n), \mathbf{\Gamma}(n), \mathbf{\Phi}_L(n), \mathbf{\Phi}_R(n))$.

    The wing kinematics array thus consists of data for both wings and each combination of video frame and kinematics angle. For example, the kinematics of the left wing is defined using a set of three arrays as $\boldsymbol{\phi}_L(n) = (\boldsymbol{\phi}_L(n), \boldsymbol{\eta}_L(n), \boldsymbol{\alpha}_L(n))$.

Quantify the Leg Kinematics of Moving Mosquitoes

When also tracking leg kinematics, these data need to be added to the kinematics time–space array using the following steps:

11. Define the leg orientation using the coordinates of the leg joints and tip of each foot.

    These coordinates can be given in both the world reference frame $\mathbf{X} = \{x, y, z\}$, or similar to the wings in the body reference frame (i.e., relative to the center of the body $\mathbf{X}_{body} = \{x_{body}, y_{body}, z_{body}\}$). The specific research question will drive the choice of kinematic reference frame for the legs.

12. Add the kinematics array of each digitized leg to the complete time–space kinematics array for the digitized video $(\mathbf{t}(n), \mathbf{X}(n), \mathbf{\Gamma}(n), \mathbf{X}_{leg}(n))$.

    With six legs in total and multiple tracked points per leg (several leg joints and foot tip), the leg kinematics array can become quite large. It is therefore important to precisely and uniquely define each leg kinematics parameter.

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