SOP: Obtaining Locomotion Data from Videos

A. What this SOP covers

Use this SOP if you have recorded high-speed video of an animal moving and need to collect quantitative data from the video. This SOP focuses on running in quadrupedal animals, like lizards, that have been recorded from a dorsal view (2D) because this is the most common situation in the lab. When studying different situations, discuss what data to collect and how to collect them with Dr. Bergmann. For example, some projects may use 3D video (multiple synced videos), limb less animals, or different behaviors, such as jumping.

B. What you need before you start

- High-speed video of animal running
- Matlab with DLTdv5 and the curve fitting toolbox
- ImageJ
- Excel

C. Procedure

1. Instantaneous velocity and acceleration
    a. Instantaneous velocity and acceleration can be used to calculate average velocity and maximum velocity and acceleration.
    b. Refer to the SOPs on digitizing videos and smoothing to learn to do this.
2. Stride length and step length
    a. Variable definitions
        i. A stride is a full limb cycle, defined from foot up to the next foot up.
        ii. A stride can be divided into stance and swing. The step corresponds to the stance phase (when the foot is in contact with the ground), so from foot down to foot up. The swing phase is then foot up to foot down.
        iii. Distance traveled by the center of mass (CoM) from foot up to foot up is stride length.
        iv. Distance traveled by CoM from foot down to foot up is step length.
    b. Procedures
        i. Open the video in ImageJ (File > Open > select the video, which must be a specific format of AVI).
        ii. Play the video frame-by-frame and note the frame number for every foot up and foot down for the limbs for which you want to calculate stride and step length (generally both hind limbs).
        iii. You may need to play forward and backward to get the correct frame.
        iv. In excel, record the foot up and foot down frames for each frame. Strides should be equivalent if the animal is moving at steady speed, but if you are recording from a standstill, then strides will differ from one another as the animal accelerates and should be treated separately.
        v. Use copy the cDist (cumulative distance moved) variable from your digitization and spline data for each frame that you noted.
        vi. Calculate stride length as cDist for foot up for stride n+1 minus cDist for foot up for stride n.
        vii. Calculate step length as cDist for foot up minus the previous foot down for each stride.

3. Stride and step duration, and stride frequency
    a. Variable definitions
        i. Stride duration is the length of time the stride lasts.
        ii. Step duration is the length of time the step lasts.
        iii. Stride frequency is the inverse of stride duration (i.e., 1/(stride duration)).
    b. Procudure
        i. Open your video and note foot up and foot down frames for each stride (follow steps 2.b.i. to 2.b.iv.).
        ii. Calculate stride duration as the frame number for foot up for stride n+1 minus the frame number for foot up for stride n and divide by the frame rate (often 250 or 240 fps).
        iii. Calculate stride frequency as 1/(Stride duration).
        iv. Calculate step duration as the frame number for foot up minus the frame number for the previous foot down and divide by the frame rate.
        v. Note that step frequency doesn't make much sense because the step doesn't encompass all of the time moving (stride does).

4. Duty factor
    a. Duty factor is the proportion of the stride duration encompassed by the stance phase.
    b. Calculate duty factor by dividing step duration by stride duration.

5. Body and limb angles
    a. Definitions
        i. The angles formed by points painted on the lizard allow you to quantify how much the limbs are protracted and how much they are retracted. You can also calculate the angle to which the body (back) is bent during locomotion.
        ii. Front limb angles are calculated as the angle formed by the points on the occiput, pectoral girdle, and elbow. The minimum angle is the angle of maximal protraction, and the maximum angle is the angle of maximum retraction.
        iii. Hind limb angles are calculated as the angle formed by the points at the cloaca, pelvic girdle, and knee. The minimum angle is the angle of maximal retraction, and the maximum angle is the angle of the maximum protraction.
        iv. The body angle is calculated as the angle formed by the points at the pectoral girdle, the mid-back, and the pelvic girdle. When the back is straight, this angle is 180_o. Alternating minimum angles are the maximum bend to the left and right.
        v. Note that many other angles could be calculated, but are dependent on having points painted in the right places.
        vi. Also note that using 2D video analysis to calculate angles assumes that the movements of the parts of the animal occur in a plane (2D). If this is not the case, then it may be necessary to record the animal in 3D, using two or more synced cameras.

    b. There are two ways to calculate these angles.
        i. One approach requires that all of the points are digitized using DLTdv5 in Matlab (see appropriate SOP to do this) and the angle formed by any three points is calculated for every frame. This approach is likely more accurate because it allow selection of

maximum and minimum angles from all frames. This approach is much more time consuming.

ii. The other approach requires that the researcher identify the frame in which an angle is maximal or minimal and calculates the angle in ImageJ only for the frames needed. This is a much more efficient approach, but may lead to slight error if it is difficult to identify exactly which frame has the maximal or minimal angle.

c. All frame procedure

i. Open the comma-delimited file containing the coordinates for all digitized points that you produced using DLTdv5 in Matlab by double-clicking on the filename. This filename should end in "xypts.csv".

ii. Open the Excel spreadsheet "Angle Calculation Sheet.xls".

iii. Follow the instructions in the Excel spreadsheet.

iv. You will need to highlight the columns with the x and y coordinates for each of the three points of interest in the comma-delimited file and paste them into the appropriate columns in the Angle Calculation Sheet.

v. Fill down the contents of the five right-most columns of the Angle Calculation sheet to match the length of your pasted coordinate date. The equations in these last five columns will automatically calculate the angle for every frame in your video i the right-most column.

vi. Go to the bottom of the Angle Calculation Sheet and below the last cell of the right-most column, type "=max(" and highlight all cells in the column that have numbers in them. Press <Enter>.

vii. Repeat step C.5.b.vi., but type "=min(".

viii. Transcribe the max and min angle values to your own spreadsheet that contains the data for your project. Be careful to ensure that you understand the biological meaning of the min and max values. They can change depending on whether the animal is running left to right or right to left.

d. Selected frame procedure

i. Open your video in ImageJ, following steps C.2.b.i to C.2.b.iii.

ii. Play the video frame-by-frame, forward and backward to identify the frame in which the limb of interest or the body (or whatever is of interest) forms the maximum or minimum angle.

iii. Click on the "Angle Tool" button, which is the sixth from the left.

iv. Click on the three points from which you want to calculate the angle on the frame of the video, with the second point being the vertex of the angle (where the two legs of the angle meet).

v. Press <ctrl> and <m>, for "measure".

vi. A results window will pop up and one of the numbers will be the labeled as "Angle". Copy the angle number into your spreadsheet.

vii. Repeat steps C.5.d.ii. to C.5.d.vi. for all angles you wish to calculate in your video.