Fly Walking Tracking Protocol

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This should be done in two stages. First, open the video to determine which frames will be tracked, determine the spatial scale, and measure the body length and leg length. Then close the video and reopen it only with the frames of interest to do the tracking. Also, you can install a few simple macros to eliminate some of the more tedious calibration steps. The StartupPacros.fiji.ijm file is in the “code” folder. Copy this over your current startup macros file.

1. First, determine which frames will be tracked.
   1. Open the video in ImageJ
   2. Find the frame where the first leg moves, two frames before that is your start frame. Enter this number in the spreadsheet
   3. Find the frame where the last leg moves for the last time, two frames after it plants that leg for the last time is the end frame. Enter this number in the spreadsheet.
2. Perform spatial calibration (real distance of each pixel)
   1. Select the line tool and draw a line across the scale bar. You should include the full thickness of the beginning line, and end at the start of the end line.
   2. Go to analyze -> set scale
   3. “Distance in pixels” should be filled out, it is the length of the line you drew.
   4. Under “known distance”, put 5.
   5. Under “unit of length”, put “mm”
   6. Enter the number that appears after “Scale:” on the spreadsheet
   7. Click ok
   8. **Alternatively:** if you have the macros installed, draw the line as in a, then type “5”. It will set the calibration and copy the number to your clipboard.
3. Record body length
   1. Find a frame where the body is straight and not scrunched up, and ideally, it is near the scale.
   2. Using the “line” tool in FIJI/ImageJ, measure the entire length of the body, from the area directly between the antennae to the posterior tip of the abdomen.
   3. Type “m” and copy the length number onto the spreadsheet.
   4. **Alternatively:** if you have the macros installed, draw the line as in b, then type “2”. It will perform the measurement and copy it to your clipboard.
   5. Before moving onto the next step, close the video
4. Reopen the video selecting only the frames of interest that you determined in step 1
5. Perform temporal calibration (time between each frame). This isn’t the real time, but it allows the code to work.
   1. Go to Image -> Properties (or ctrl + Shift +P)
   2. Under “Frame interval”, enter “1 sec”
   3. **Alternatively:** if you have the macros installed, type “1” and it will do steps a-b. Note that you need to have the ImageJ/FIJI panel in focus, not the MtrackJ panel for the macro to work.
6. Navigate to the start frame.
7. Launch MtrackJ by going to Plugins -> MtrackJ. The rest of these tracking functions you will do within the MtrackJ window
8. Start by tracking the left front leg (track 1). For videos where you’re looking at the ventral surface (underside) of the fly, find the left side by imagining you’re shaking hands with the fly. Click as close to the center tip of the foot as possible. After clicking, the video will advance to the next frame.
9. Click on the foot for every frame where it is planted (not moving or blurry) until the end frame. For frames where the leg is swinging in the air, just advance to the next frame without clicking. Do click on any frames where the foot is sliding along the surface.
10. After completing the track, press escape or double-click to end the track
11. For each of the remaining legs in the order shown below, rewind the video to start frame and repeat steps 7-9.
12. For the next track (track 7), click on the very front of the body in each frame
13. For the next track (track 8), click in the center of the body between the back two legs
14. For the next track (track 9), click on the very end of the body
15. When all tracking is complete, select “Measure” in the MtrackJ window
16. Two windows will pop up, one which is much shorter and is a summary of each track. The other one is the complete information for each point. Save this one as the filename of the video with your initials and .csv instead of .avi as the extension. Example filename- CA32\_A\_1\_1\_00-00-05\_EB.csv
17. Save the tracks in .mdf format, click “Save” in the MtrackJ window and save this file with the same filename as the video with your initials and .mdf instead of .avi. Example filename- CA32\_A\_1\_1\_00-00-05\_EB.mdf

Tips and Tricks

* If you accidentally end a track, click “Add”, then hold down control and mouse over the track of interest. It should be highlighted in white. Click the track to continue it.
* If you make a mistake in a track, click the “Delete button” and click on the point you want to delete.

