

Langerhans Lab Protocols

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High-speed Video Locomotion Analysis

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This document explains how to:

- Capture behavior on video using the HSV camera
- Measure acceleration, velocity, and displacement by digitizing videos in TPSDig2
- Use QuickSAND to smooth the locomotion data from TPSDig2

Using the HSV camera

1. Download the version of Motion Studio appropriate for your computer:
Motion Studio 32 bit
www.idtvision.com/downloads/mstudio.exe
Password is “veryfast!”

Motion Studio 64 bit
www.idtvision.com/downloads/mstudio64.exe
Password is “ALotOfBits”
2. Plug the camera into a power outlet
3. Connect an Ethernet cable between the camera and your computer.
4. Reset your computer’s IP address and subnet mask to be compatible with the camera
 - a. Control Panel-Network and Internet-Network and Sharing Center, Change Adapter settings, Click Local Area Connection, Highlight Internet Protocol Version 4, Click Properties
 - i. Computer network IP address: 10.10.10.186
 - ii. Computer subnet mask: 255.255.255.0
5. Open Motion Studio and make sure camera IP address is set
 - a. Highlight camera name in program, Click IP Address
 - i. Camera network IP address: 10.10.10.188
 - ii. Camera subnet mask: 255.255.255.0
6. Start filming

Camera specs

MAC address is: 00-25-16-00-0C-CD

Serial number is 0201110522

Help with lenses

Besides the lens that came with the camera, macro lenses in the lab can be used, including the Canon Normal EF 50 mm Compact-Macro, and the Sigma 17-70mm 1:2.8-4 DC Macro HSM. The Sigma 17-70 allows for the smallest field of view, which is useful in filming small (e.g. 2 inch) fish.

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These macro lenses attach to the camera only with the help of the lens adapter, a black cone-shaped piece that screws into the body of the camera. To attach the lens to the adapter, line up the red dots on both parts and twist them together. To release, slide back the small silver knob on the adapter and untwist the lens.

This adapter should work with any Canon lens as long as it is an EOS lens.

Digitizing videos in TPSDig2

This procedure generates displacement data from video sequences.

1. Open TPSDig2 (download available at <http://life.bio.sunysb.edu/morph/index.html>).
2. In the **File** menu, select **Input source>File**.
3. Choose the video file you want and click Open. If you can't see the file, select **All graphics** from the dropdown **Files of type** menu.
4. When you have opened the video file in TPSDig2, a "Multimedia input" window will open, from which you can watch the video. Use the arrow buttons or progress bar in the Multimedia input window to go to the first video frame you want to start with, and click **Capture**. This will cause the current video frame to open in the regular TPSDig2 window.
5. In the TPSDig2 window, click on the button with the crosshairs icon—this puts you in "digitize landmarks mode"—then click on the points in the frame you want to landmark (tip of the animal's nose, for example).
 - a. If you don't like where you put a landmark, you can move it by switching back to "Edit mode" by clicking on the cursor arrow button on the menu bar.
 - b. You can delete landmarks by right-clicking with the mouse over the landmark and selecting **Delete**.
6. When you are satisfied with the points you landmarked, go back to the Multimedia input window and use the arrow buttons to go to the next frame in the video. Click **Capture**.
7. TPSDig2 will ask you if you want to save your changed image. Select **No**.
8. Your new video frame will appear in the TPSDig2 window. The landmarks you added to previous frames will also be visible. Repeat your actions from step five.

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- a. If having previous landmarks visible is inconvenient, you can make all landmarks invisible if you are using TPSDig2 version 1. Select the **Image tools** button—the one with a hammer on it—at the top of the screen. Go to the **Colors** tab and, in the **Landmarks** group, change the **Lm color** width to zero.
9. When you have digitized all the frames you want, go to **File>Save data** in the TPSDig2 window (TPSDig2 will ask you again if you want to save the changed image. Select **No.**) You have now created a TPS document with landmark and image information. The landmarks are in x y coordinate pairs.

Making a Scale

1. Open a video file in TPSDig2.
2. Open the **Image tools** box and select the **Measure** tab.
3. Choose the reference length you want (e.g., 3 centimeters), then click **Set scale**.
4. In the TPSDig2 window, click on your reference points (e.g., the two endpoints of a 3 cm line).
5. Go back to the **Image tools** box and click **OK**. The scale line you created will disappear, but its value will be registered in the TPS file you are about to create.
 - a. If you want to redo your scale measurement, you can just click **Set scale** again, re-measure your reference length, and click **OK**.
6. Go to **File>Save data**. (TPSDig2 will ask you again if you want to save the changed image. Tell it **No.**) This will create a .TPS document with scale information.
 - a. Alternatively, you can set a scale at any time while digitizing video sequences. The scale information will appear in the TPS file at the bottom of the landmark list for the frame you set the scale in.

Generating Displacement Data

1. Open the TPS file you created. You will see a set of progressively longer lists of landmarks.
2. Delete everything above the last, longest list.

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3. Save and close the TPS file.
4. Open Microsoft Excel.
5. Go to **File>Open**.
6. Open the TPS file you just created (Hint: select **All files** from the **Files of type** menu).
7. A Text Import Wizard box will open. Under “Original Data Type,” make sure **Delimited** is selected. Click **Next**.
8. Under **Delimiters**, check the box next to **Space**.
9. Click **Finish**. You should now have all your landmark coordinates in two columns in your Excel file (left column is X coordinates, right column is Y coordinates).
10. Use Excel to multiply each coordinate by the scale value from your TPS file.
11. Using this new list of “scaled” coordinates, find displacement by taking the square root of the sum of the squared difference between scaled coordinate pairs, i.e.—
$$\sqrt{((X_1-X_2)^2 + (Y_1-Y_2)^2)}$$
12. A sample Excel template for calculating acceleration, displacement, and velocity from TPS files can be found under the Langerhans Lab Protocols folder (file name: “Sample template TPS to locomotor data.doc”).

Shortcuts

1. When you want to move to and capture the next frame of the video you are working on, go to the Multimedia input window, click the forward arrow button that takes you to the next frame, then hit the space bar (for capture) followed by the “n” key (for “no”). You actually don’t have to activate the Multimedia window before you click the arrow button: if you’ve been working in the TPSDig2 window, moving your mouse to the Multimedia window and clicking the arrow button will both activate the window *and* advance the video one frame.
2. You can switch between “landmark mode” and “edit mode” in the TPSDig2 window by right-clicking with the mouse instead of using the buttons in the menu bar.
3. When the Text Import Wizard box appears as you are opening a TPS file in Excel, typing “nsf” will give you the same results as following steps 7–9 above.

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Smoothing data in QuickSAND

This procedure takes the displacement data you generated in the last step above, and generates smoothed acceleration and velocity values using your estimate of error.

Measure mean squared error

1. Digitize the same video twice
2. Measure your mean squared error by finding the average variance in scaled displacement for each time step. An example of this can be found in the Langerhans Lab protocol folder, in the file named “Finding measurement error for QS.xlsx”
3. Repeat for other video(s) and take the average of your mean squared error for multiple trials.

Import data into QuickSAND

1. From following the steps in “Generating Displacement Data” above, you can create an Excel file for each video, with three columns for cumulative time, cumulative displacement, and step displacement (example below). The column order is important.

0	0.110918	0.110918
0.004	0.286295	0.175377
0.008	0.397213	0.110918
0.012	0.61905	0.221836
0.016	0.901837	0.282787
0.02	1.184624	0.282787

2. Open the Excel file. In the File menu, select Save As.
3. From the “Save as type” dropdown box, select “Text (Macintosh)”
4. Open QuickSAND on the older Mac computer.
5. Check the box next to **Optimally Regularized Smoothing Spline**. Leave all other boxes unchecked.
6. In the Optimization field, select **MD=3 Prior given VAL (error variance)**

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7. In the Spline Order field, select **M=3 Quintic spline**
8. Leave the Smoothness field set to zero.
9. Enter your average mean squared error (from above) in the Error (VAL) field.
10. Select File- Open and open the Macintosh text file you made in step 3. A graph of unsmoothed displacement values (Y) will appear on the screen
11. Click the button labeled **Do it**
12. Select **Do it** again. Graphs of smoothed displacement (Yhat), velocity (dY/dt), and acceleration ($d(dY/dt)/dt$) will appear.
13. In the File menu select “Save as” and choose a name for your output file. This file will have four columns: time, displacement, velocity, and acceleration. Select Save.
14. In the File menu select “Close.” This clears the screen so you can open another file.