**Jung, S. H., Hueston, C. &amp; Bhandawat, V. Odor-identity dependent motor programs underlie behavioral responses to odors. Elife 4, doi:10.7554/eLife.11092 (2015).**

This document provides some instructions on how to run the code associated with the manuscript above. The attached code performs two main tasks:

1. Given two views of the behavioral arena, it registers these two views to obtain a single view from the top of the arena. Using this view, it performs background subtraction and tracks the fly.

2. Using the tracks, the code performs some analyses on a single fly and some analyses on multiple flies to perform group analyses.

**Behavioral Video Registration**

1. Run **“batch\_registration\_bgs\_tracking\_V10\_130110\_for\_T3600.m”** (under **“mfiles” folder**) on MATLAB.
2. **“Which video to start?”**

Type in the trial number corresponding to the video you want to analyze (ex. On the GUI, the trial # read: “31” → type “31”). This is the first video number.

1. **“Until which video?”**

If you are analyzing only one video, this is the same number as the first video number. (ex. Type “31.”)

If you are analyzing all of the trials that you ran that day, type the number of the last trial (ex. Type “33.”)

When prompted, select the first camera view of the **first** video and *then* the second camera view of the **first** video (ex. Select “160629video31\_**1**” and then select “160629\_video31\_**2**”)

1. **“Type in the frame number where the fly is inside the odor zone:”**

Open one of the videos in QuickTime. Find a point in the video where the fly would be clearly visible in both videos (preferably in the center of the chamber). Click on the time display in the lower left hand corner of QuickTime Video Player display and select “Frame Number.”

Type this number into the MATLAB prompt.

1. **“If you want to re-use the old registration information, press 1, if not, press 2:”**

Type 2. A file explorer window will now appear. Select “**151006ref\_tform**” from the “**151006\_registration**” folder. .

1. In the next windows, you will need to select the fly’s head in the designated frame number from both camera views. *Make sure to select the SAME point in BOTH views -- if you don’t, the resulting image will be distorted.*
2. **“To apply an existing mask, press 1, otherwise 2.”**

Type 2.

1. A new window will pop up. **“Click on the image at the height at which you would like the image to be between the two cameras. Then press RETURN.”**

Click on a midpoint -- this will act as a horizontal line of separation between the two camera views in the registered image.

1. **“If you want to see the registered image of reference paper, press 1, if not press Enter.”**

Type 1.

1. **“If you want to save the file, type in the name (excluding date, string only).”**

Type in a file name (ex. Video31\_merged).

1. **“Have you already saved rim points for this date? 1 for yes, 2 for no.”**

Type 2. Follow the instructions. BE VERY PATIENT WITH THIS STEP. The ellipses can be very slow to expand and even more difficult to move around when they are stacked on top of or in close proximity of each other.

Tip: Create all ellipses of appropriate sizes and *then* rearrange the ellipses in their appropriate locations (don’t worry if you double click after creating the first three ellipses and the first three ellipses aren’t in their appropriate spots -- you can adjust ALL ellipses’ positions when you are setting the fourth and final ellipse).

1. At this point, the program will run for a good chunk of time (>20 minutes). You should see     “startframe = \_\_” appear frequently. Don’t be alarmed if you see “if loops” too -- let the program run its course.

**It would be smart to open multiple MATLAB windows (no more than 3 or 4 though) to start registration of your other trials while this “startframe” business is running.**

1. After the program finishes running, it will have produced a list of “frames to correct.”

**Framechecker**

1. Open **“framechecker.m”** (found under **“mfiles”**).
2. Type “[frames\_to\_correct] = framechecker(trial #, trial #)” in the command window.

**In case you copied and pasted that tidbit word for word into MATLAB, don’t forget to replace “trial #” with the actual number of the trial you’re working on.**

Hit Enter.

1. A DLTdv5 controls window will pop up. Hit “Initialize.” Select “1” for # of videos to analyze. Select your background subtracted video for the trial.
2. Hit “Load Data.” Select the Excel file with “xypts.” You should see a red circle form around your fly in the Movie window.
3. In the “Workspace” section on MATLAB, double click “frames\_to\_correct.” Scroll all the way to the right until you see the last cell with a matrix of numbers. Double click the cell. You can now see all the frames that you need to correct in your registered video in a column.
4. Go through each of those frames and correct them. You’ll find that in the list of frames you need to correct, you’ll often be given one or two frames before and after the frames that truly need to be corrected. Use those as guides to correct the frames that show the fly to have almost teleported to a completely different (obviously WRONG) location than indicated by their path in the before and after frames. You may end up clicking the same spot for a sequence of frames if the fly doesn’t move during that time period.

**Use the registered video to verify the fly’s locations at specific frames when correcting the frames. You’ll find that the majority of the frames needed to be corrected are the ones where the fly is near the inner and outer rims of the arena or near the horizontal line of separation in the registered video.**

**It may seem tedious and like a lot of clicking, but don’t be surprised if your first few trials give you ~2000 frames to correct. Always be thinking of ways you can reduce this number for future trials. It may be as simple as adjusting the black platform that the flies walk on so that all areas of the arena are accounted for in the camera views.**

**Transformer**

1. Run **“transformer.m”** (found under **“mfiles”**).
2. **“What is the first video?”**

Type trial #.

1. **“What is the last video?”**

Type trial #.

4. **“Do you want to re-use the transformation matrix saved previously? 1 = Yes, 2 = No”**

Type 1. Select “**151006ref\_tform**” from the “**151006\_registration**” folder.

**Single Fly Analysis**

1. **“If you want to repeat this analysis with exactly the same parameters, press 1, otherwise 2.”**

Type 2.

1. **“What is the first video?”**

Type trial #.

1. **“What is the last video?”**

Type trial #.

1. **“What is the fly genotype?”**

Type in the fly genotype you are working with (ex. Rut1control, rut1mutant, etc.).

1. **“If you have already saved the odor names, press 1, otherwise 2.”**

Type 2.

1. **“What is the odor name and concentration for video\_\_?”**

Type in the odor used for that trial (ex. H2O, ACV2, ACV).

1. **“Do you need only main analysis or the whole thing? If you need only the main analysis, press 1, otherwise type Enter.”**

Type 1.

1. **“Do you want to re-use the saved run/stop determination info? If yes, press enter, if no, press 1.”**

Type 1.

1. **A graph will pop up. Look at the first point on the y-axis. If it is not 0, this is considered a RUN.**
2. **“Type 1 if the first point is a run, 0 if a stop.”**

More than likely, type 1.

1. **A PDF showing the analysis for that trial should pop up. It may close abruptly because the program will automatically save the PDF. “Pdf file saved!”**

**Multi Fly Analysis**

1. Create a folder for each conditions: such as genotype + odor combination

2. For each experiment we need a *.csv* file which contains the xy coordinate + *.mat* file which contains the coordinates for the inner and outer rim.

3. run ‘multiple\_flies\_analysis\_V22\_biggerIR.m’ : run IR=1.2 or 1.9

4. run ‘multiple\_flies\_analysis\_V22\_VB\_combined.m’. This seems like a repetition of step 3, but it extends the analysis performed in Step 3. The way this code was written, it makes it necessary to do step 3 before 4 because step 4 needs a .mat file generated in step 3.

5. run ‘Datacollector.m’ . This step saves final parameters (before/during compared) as double arrays and created two .mat files.

\*data.mat and \*distribution.mat

6. To compare across multiple conditions multiple groups, use ‘group\_comparison\_plots.m’

First, copy \*data.mat and \*distribution.mat to a different folder where all different genotype+odor are saved.

Type in fly genotypes and odors.

This will plot one figure that shows all the behavioral parameters + radial density plot.