Steps for Conducting Geotaxis Experiments:

1: Load desired flies in the 14-inch plastic tubes.

\*\*\*Make sure to keep track of what genotype they are.

\*\*\*Make sure the plastic cap with the holes is not covered. Important to allow the flies to breathe.

2: Load the tubes into the designated locations in the “slammer” device, aka, the acrylic mount.

A close-up of a white tube

Description automatically generated

3: Write a description text file saved to the same folder with the date, how old the flies are, what gender they are, and what genotype is, if there are any drug feeding concentrations to keep track of, in which position, per each experiment.

A screenshot of a computer program

Description automatically generated

4: Make sure the slammer device and camera are plugged in. If this is the first time using the slamming device, make sure the following are downloaded.

- MATLAB anything before v2022 along with the following toolboxes

(\*\*\*2023 version does not allow im2frame() function to work with the TisImaq to work---for the camera)

* computer vision toolbox
* data acquisition toolbox
* database toolbox
* image acquisition toolbox
* imaging processing toolbox
* instrument control toolbox
* parallel computing toolbox
* statistics and machine learning toolbox

\*\*\* make sure the folder with all the functions for the slammer are added to the path in MATLAB

-[IC Matlab Plugin for Matlab R2013b and Newer Versions 3.4.0.58 (theimagingsource.com)](https://www.theimagingsource.com/en-us/support/download/icmatlabr2013b-3.4.0.58/)

\*\*\*follow the instructions after file is downloaded and installed

\*\*\*Once the plug in is registered using the matlab file downloaded with the folder, make sure to clear all in the command line.

A computer screen with text

Description automatically generated

-[BenchVue Basic Offline Installer Download | Keysight](https://www.keysight.com/us/en/lib/software-detail/computer-software/benchvue-basic-offline-installer-download.html)

- [IO Libraries Suite Downloads | Keysight](https://www.keysight.com/us/en/lib/software-detail/computer-software/io-libraries-suite-downloads-2175637.html)

5: Plug in the wall of LED lights on the back of the slammer then turn off all the lights in the room. Make sure the only lights that are pointed at the vials are the LED wall lights. That means making sure the computer screen is pointed away from the vials.

6: Open MATLAB, when you are ready to begin the experiments use the **RecordVideos().** It will ask you to input the following information with default values.

A screenshot of a video form

Description automatically generated

When satisfied with your inputs, click the OK button to start.

\*\*\*Try not to walk around while the camera is recording. It could result in shadows on the vials that may disrupt fly tracking.

ONCE THE EXPERIMENTS ARE COMPLETE AND YOU ARE READY TO ANALYZE

7: For the first video, use **VideoProcessor**([],#\_of\_vials\_in\_exp,3,1). Click the first video in the experiment. Then specify the regions of interest (ROIs). Do this by clicking the top left corner of the vial and drag to the bottom right corner.

\*\*\*if you mess it up, it will allow you to edit after you draw all the ROIs expected (specified in the command line by the user). After all are drawn, any adjustments can be made.

Once satisfied with the ROIs, click “Enter” on your computer keyboard. It will save new videos using the ROI, creating vial videos saved directly to the folder in use.

A picture containing chart

Description automatically generated A black specks on a white surface

Description automatically generated

8: For convenience, load the ROI.mat file into your workspace and call **VideoProcessorIterative**(roi,#\_of\_vials\_in\_exp,3). Select the rest of the videos. It will automatically use the ROI created from the first video and cut the rest down to size saving to the same folder.

8.5: If this is a new set up, I highly encourage the user to calibrate the tracking. **Calibrate**(0,1) for the first vial video calibrated, for the rest **Calibrate**(1,1) allowing you to append to the existing excel files. Sort the detected objects in each vial video as a single fly, multiple flies, or noise.

A close-up of a white pillar

Description automatically generatedA close-up of a white pillar

Description automatically generatedA close-up of a rectangular object

Description automatically generated\*\*\*50-100 vials are recommended. Preferably if each location on the slammer has the same number of representative videos.

9: FlyTracking=([List\_of\_#\_of\_flies\_per\_vial], 1, 0, 1, 10, number\_of\_vials). Input all vials from the same experiment when prompted.

\*\*\*I suggest checking the first few videos to make sure the tracking is accurate for the videos.

A group of yellow paper strips

Description automatically generated with medium confidence

10: Once all the coordinate cell matrices are complete use groupTracksPerVial() function to separate the vial matrices to be used in analysis. If user needs to combine vials of the same genotype together, use combineVials() to do this action. combineVials() can also be used to combine vials from different experiments together. The analysis desired is up to the user.

11: Use info=Geotaxis\_genotypecompare\_V5() to analyze data. \*\*\*save the info structure output as well as the output figure to the folder.