**PySolo-Video:**

* Start “Spyder” and run the “pgv.py” script.
* If you have a configuration to load, do so, or make changes to the configuration now.
  + In “Option > Configure”, set the output data folder location.
  + You can also change different settings like FPS, how many monitors/videos you want to be displayed, size of these thumbnails, etc. This only affects what you will see in the PySolo program, not how the data will be processed. Save when finished.
* On the left side of PySolo-Video window there are two tabs: “Thumbnails” and “Live View”. You are in the Thumbnails panel by default when PySolo-Video is launched.
* In the thumbnail view,
  + Select a thumbnail monitor.
  + Use “Select Video Input > File > Browse” to find the movie that will play on that monitor.
  + “Apply”
  + “Play” if you like, then stop the video before proceeding.
* In the live view,
  + Select the monitor number from the dropdown menu.
  + The movie name will appear below the dropdown menu and the movie will play.
  + If you will be using an automatically generated mask, use the “maskmaker.py” program to generate the mask before proceeding.
    - Load the mask and make sure it fits correctly. If not, make adjustments to the maskmaker settings and run it again.
    - You can clear and reload the mask as many times as you need to.
  + If you will be creating the mask by hand, define ROIs for each well with a fly in it.
    - To define an ROI and start tracking a fly, left click and drag around the well.
    - A red rectangle will be drawn for your selection.
    - Inside this rectangle, double click the left mouse button to create the ROI.
    - Fly tracking in this ROI will be displayed. Each fly will have a small red rectangle drawn on it and white + sign and you will be able to see it moving as the fly moves.
    - You may de-select the most recently drawn ROI by right clicking anywhere inside it. You may de-select all the ROI by clicking the “Clear All” button in the Mask Editing section displayed below the monitor number.
    - The order in which you draw the ROIs is the order in which the data will be written in the final file. The ROIs are analogous to channel numbers in DAM.
  + Once all the ROIs for all the wells with flies are drawn, the “Save and Apply” button under Mask File section. You will be prompted to save the file where you can change the name of the mask file (.msk file).
  + You can apply any mask to any other monitor, even for a different video file, by using the “Load Mask” button.
  + If you create more than 32 ROIs in the mask, additional manipulation of the output file will be required after running PySolo Acquire.
* Return to the Thumbnails panels (tab on the left side)
  + Under “Set Tracking Parameters” choose the mask file that you just created in the Mask File section.
  + Under “Calculate Fly Activity as…”, choose “Activity as Distance Traveled”.
  + Click and check “Activate Tracking”.
  + Make sure the initial video file and the associated monitor number are correct.
  + If everything looks right, click “Apply” in the Select Monitor section.
* Click “File > Save as” and save this entire configuration under a recognizable name (.cfg file). Click File > Exit program to close pySolo-Video.
* You will need to edit the config file that was created…

**PySolo-Acquire:**

* Before starting the program, you need to edit “pysolovideo.py”.
  + Use “find” to search for “Datetime settings”
  + Change the date and time assigned to “real\_dt” to match the start date and time of the video.
  + Save the changes.
* Run “pvg\_acquire”.
  + Browse to load the PySolo configuration file that you just created in PySolo Video.
  + You will see a table with the monitor number, source video, associated mask file, output file name, and tracking type for each monitor (Track type 0 = Activity as Distance Traveled).
  + Check the monitor(s) you want to acquire data from.
  + Click the “Start” button at bottom left of the window.
  + A “.txt” file will be created and PySolo-Acquire will start writing movement data into it.
  + The program does not indicate completion, but when the date-time of the output file is no longer the same as the current date-time, processing is compete and PySolo Acquire can be stopped and closed.

**Excel manipulation for data with more than 32 ROIs:**

* Open the file in Excel.
* The Text Import Wizard will be displayed.
  + The file type is “Delimited”. Click “next”.
  + The delimiter is “Tab”. Click “next”.
  + In the “Data preview” table at the bottom, select all of the columns.
  + Change the “Column data format” to “Text”. Click “Finish”.
* For each set of up to 32 ROIs, open a new spreadsheet.
* Copy columns A-J into each of the new spreadsheets.
* Copy the first 32 columns (K-AP) into the first spreadsheet at column K.
* Copy the next 32 columns into the second spreadsheet at column K.
* Continue until all columns are in their own spreadsheet.
* Any unfilled columns in columns K-AP should be filled with zeros.
* Save each Excel sheet as a text file with the name in the format “Monitor##.txt”. Use leading zeroes for the numbers 1-9.

**Convert the modified .txt files by using normal DAMFileScanner software.**

**Create graphs using SCAMP**