***Manual Paw Joints Marking Protocol***

1. Make sure you can open NAS4. If not, open the Run program and type in ***\\172.20.138.143\RecordingsLeventhal04\***. You may have to login with your NAS login info (check your email).
2. Open MATLAB and navigate to your GitHub folder. Make sure the GitHub folder (with the Skilled Reaching repository inside) is added to the path (*right click GitHub folder in Current Folder tab 🡪 Add to path 🡪 Selected folders and subfolders*). If errors do occur during paw marking, they will appear here, in the command window of MATLAB.
3. Type ***createManualPawData\_2015\_06\_19*** in command window of MATLAB and hit enter. This will initiate the paw point marking programs. Though all variables created will be saved along the way, **all the important data** (e.g. the manually determined start or trigger frame, the coordinates for each marker for all the frames of a video, images of the marker placements in every frame marked) **is being saved to the RatData structure, see full details in the table below:**

|  |  |
| --- | --- |
| Important Data | Location in RatData Structure |
| Filepaths to raw data for every session/date | RatData 🡪 Date Folders  Also contained in variable AllRatDateFolders |
| Information about each of the video files taken for each trial in a given session/date (name of file, date of creation, # of bytes, etc.) | RatData 🡪 VideoFiles |
| Videos in format that MATLAB can read (VideoReader Objects) | RatData 🡪 VideoFiles 🡪 Object |
| Manually determined start frames for each trial/video | RatData 🡪 VideoFiles 🡪 ManualStartFrame |
| Number of frame in which every marker is placed within a video file | RatData 🡪 VideoFiles 🡪 Paw\_Points\_Tracking\_Data 🡪 Column 2  Also:  RatData 🡪 VideoFiles 🡪 Paw\_Points\_Frame\_Data 🡪 Column 1 (each row contains data for a different frame in Paw\_Points\_Frame\_Data, more info below) |
| Region of frame in which every marker is placed within a video file | RatData 🡪 VideoFiles 🡪 Paw\_Points\_Tracking\_Data 🡪 Column 3 |
| Number of marker out of all the markers placed in a given frame region (i.e. out of 16) | RatData 🡪 VideoFiles 🡪 Paw\_Points\_Tracking\_Data 🡪 Column 4 |
| Finger in which a given marker is placed in all frames of a video file (except for Pellet Center and Center of Back of Paw) | RatData 🡪 VideoFiles 🡪 Paw\_Points\_Tracking\_Data 🡪 Column 5 |
| Anatomical names of every marker placed in all frames of a video file | RatData 🡪 VideoFiles 🡪 Paw\_Points\_Tracking\_Data 🡪 Column 6 |
| Coordinates of every marker placed in all frames of a video file (one per trial)  (If the marker is not visible, it will appear as ‘NaN’, i.e. Not a number, here. If it was never placed, like if an error occurred, it will appear as ‘Marker Not Yet Placed’) | RatData 🡪 VideoFiles 🡪 Paw\_Points\_Tracking\_Data 🡪 Columns 7 (x-coordinate in pixels) & 8 (y-coordinate in pixels) |
| Frame order for all markers placed in a video file | RatData 🡪 VideoFiles 🡪 Paw\_Points\_Tracking\_Data 🡪 Column 9 |
| Frame region order for all markers placed in a video file | RatData 🡪 VideoFiles 🡪 Paw\_Points\_Tracking\_Data 🡪 Column 10 |
| Image of frame at last marking (should match RatData 🡪 VideoFiles 🡪 Paw\_Points\_Frame\_Data 🡪 Column 58 if completed correctly) | RatData 🡪 VideoFiles 🡪 Paw\_Points\_Frame\_Data 🡪Column 2 |
| Path to MATLAB figure of cropped image for a given frame (with just the zoomed in images, used for marking paw points) | RatData 🡪 VideoFiles 🡪 Paw\_Points\_Frame\_Data 🡪 Column 3 |
| Zoomed-in images used in placing markers for a given frame and positions of those images in the larger frame | RatData 🡪 VideoFiles 🡪 Paw\_Points\_Frame\_Data 🡪 Columns 4-9 (4: left mirror image, 5: left image position, 6: center image, 7: center image position, 8: right mirror image, 9: right image position) |
| Images of frames analyzed in a given video file (without markers placed) | RatData 🡪 VideoFiles 🡪 Paw\_Points\_Frame\_Data 🡪 Column 10 |
| Images of frame with all markers placed up to the most recent marker (see order below)   1. Pellet center 2. Lunate-capitate-hamate joint 3. Thumb metacarpal-proximal phalanges joint 4. Thumb proximal-distal phalanges joint 5. Index finger metacarpal-proximal phalanges joint 6. Index finger proximal-middle phalanges joint 7. Index finger middle-distal phalanges joint 8. Middle finger metacarpal-proximal phalanges joint 9. Middle finger proximal-middle phalanges joint 10. Middle finger middle-distal phalanges joint 11. Ring finger metacarpal-proximal phalanges joint 12. Ring finger proximal-middle phalanges joint 13. Ring finger middle-distal phalanges joint 14. Pinky finger metacarpal-proximal phalanges joint 15. Pinky finger proximal-middle phalanges joint 16. Pinky finger middle-distal phalanges joint   Then repeated 3x for each region of the frame (left, center, right), so 48 markers/images total | RatData 🡪 VideoFiles 🡪 Paw\_Points\_Frame\_Data 🡪 Columns 11 to 58 |

Figure Data Contained in RatData Structure

1. Follow the prompts that appear:
   1. Select the rat’s raw data folder (RatID – rawdata) from NAS4. *Ignore any orange-colored warnings that may appear; these result from saving MATLAB’s formatted version of the video file (i.e. VideoReader format).* If this is your first time marking points for this rat, MATLAB will load the file paths to all the session folders into the RatData structure and display its progress in the command window as it does so.
   2. Select the session (a.k.a. the date) you would like to analyze. *Ignore any orange-colored warnings that may appear; these result from saving MATLAB’s formatted version of the video file (i.e. VideoReader format).* If this is your first time marking points for this rat, MATLAB will load all the videos, one for each trial, into VideoReader format (the format it needs to load videos later) and display its progress as it does so.
   3. Select the video (a.k.a. the trial) you would like to start analysis from. *This is useful if you want to stop marking close out of the program in between and resume at a later time.* ***Please finish the markers for a video completely before closing out of the program however. If you don’t, you will have to start from the beginning of that video when you go to resume it later.***
   4. Open the video file that appears and determine the start frame. *Keep the video open but minimized as it will be useful to refer to the video when doing the paw joints marking later.* 
      1. Most video files have already had their start frames determined, thanks to Maya☺. If you navigate to the session folder in the rat’s processed data folder in NAS4 (ex. For rat R0027’s data from 05/13/2014, this would be at *\\172.20.138.143\RecordingsLeventhal04\SkilledReaching\R0027\R0027-processed\R0027\_20140513a*), you’ll see a .csv file that starts with ‘*Quant Scoring’*. You can open this file in Excel and find a column labeled ‘*Start of Reach’*, usually in the first sheet, which has all the start frames listed for all videos for that day. If you can find the start frame this way, skip the next numbered item below (ii, go to iii).
      2. Alternatively, if you cannot find the start frame this way, simply copy and paste the file path displayed into Windows Explorer to open the video file in QuickTime and determine the start frame (see Figure 2 below). You’ll have *to right click* on the timer in the bottom left corner and change it to *Frame Number*, then use the indicator that indicates where in the video you are to slide to the start frame. This is the first frame in which all of the rat’s paw (i.e. all the green marked area) can be seen to breach the slit. It’s easiest to see this in one of the side mirrors: left if the rat’s dominant paw (the one marked in green) is right, and vice versa. If the rat isn’t using his dominant paw, estimate the start frame to the best of your ability.

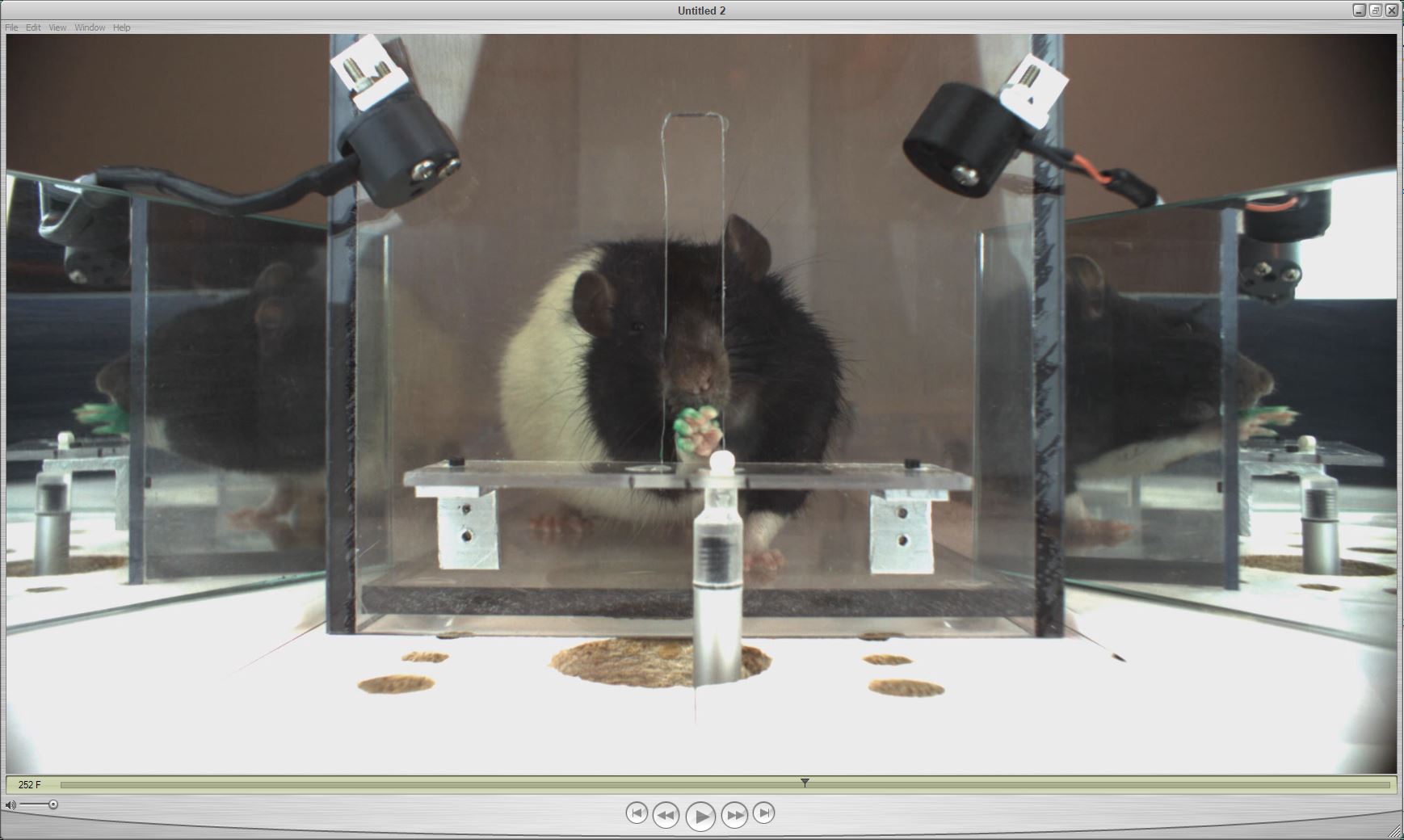


Figure Start Frame for R0027 05/13/14

* + 1. Once you’ve determined the start frame, type it into the dialog window that appeared previously and hit **Done**. If, for some reason, a start frame cannot be determined, hit **No Start Frame** (be warned that this will lead to error later on though, and you will not be to get paw point data).

1. The program for obtaining paw point data will appear in a window on the right side of the screen (the paw marking GUI). ***READ THE INSTRUCTIONS CAREFULLY.*** 
   1. First it will ask you to place rectangles around the regions of the frame you would like to zoom to in order to place markers on the paw joints. *Please do these carefully, as is not possible to redo this once it is set for a given frame.* It’s a good idea to be fairly generous here (i.e. don’t make tight rectangles around the paw and pellet, see Figure 3 below) so it is not too closely zoomed in when you go to place markers. The program will then create a figure with just the images contained in those three boxes. ***BE CAREFUL NOT TO CLOSE THIS WINDOW***, the rest of the program depends on it being open. Let the program open and close this window (and, later, the overall marked image window) on its own. If MATLAB encounters an error during this step, it should notify you and prompt you to redo the rectangles.

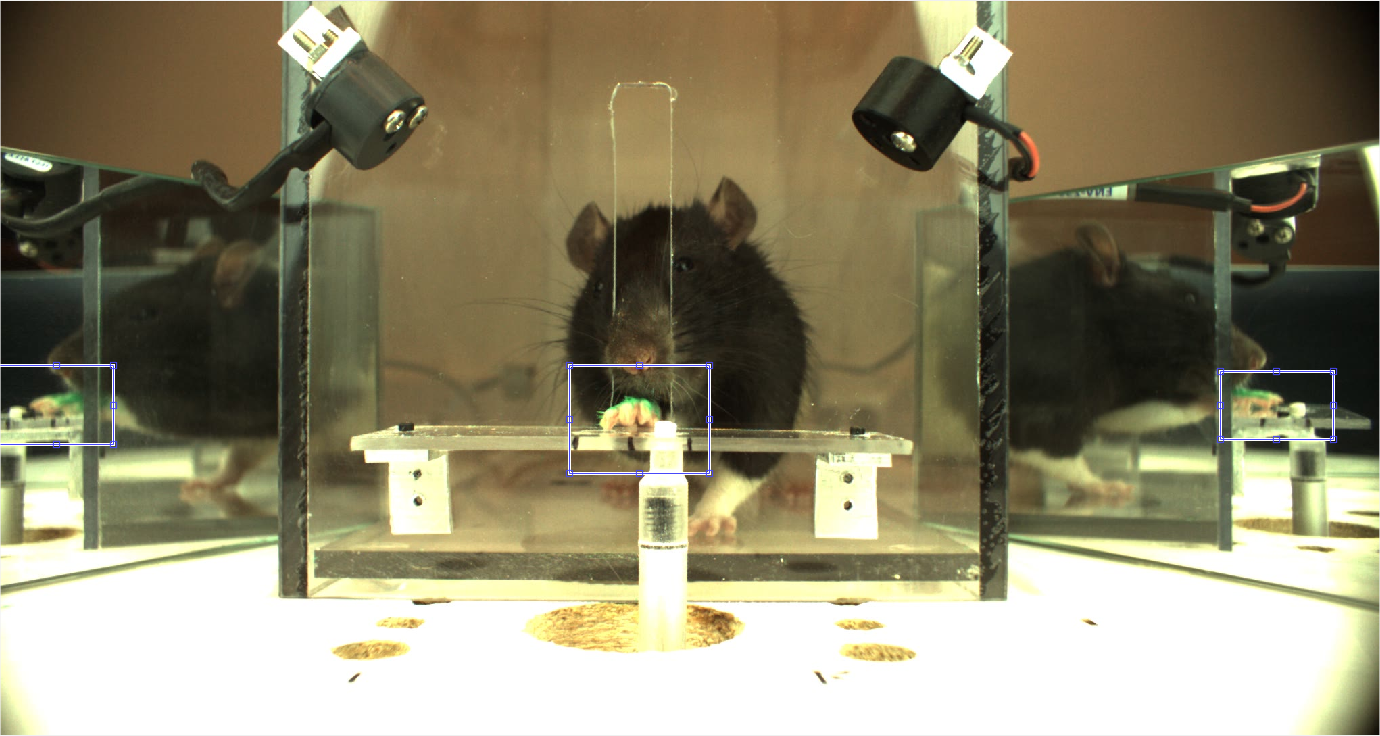


Figure Where to place rectangles for zooming

* 1. The program will then tell you which marker to mark when (the pellet center is always 1st) in the paw marking GUI window that appeared first. Please use the image that follows as a reference. MATLAB will also display important information about the marker you are currently working on in the Command Window, i.e. the marker’s name (finger + anatomical name for joint), the frame number and frame region it is contained in, and the number of this marker out of the total number of markers for the video. This will be useful if you need to redo the marker later.

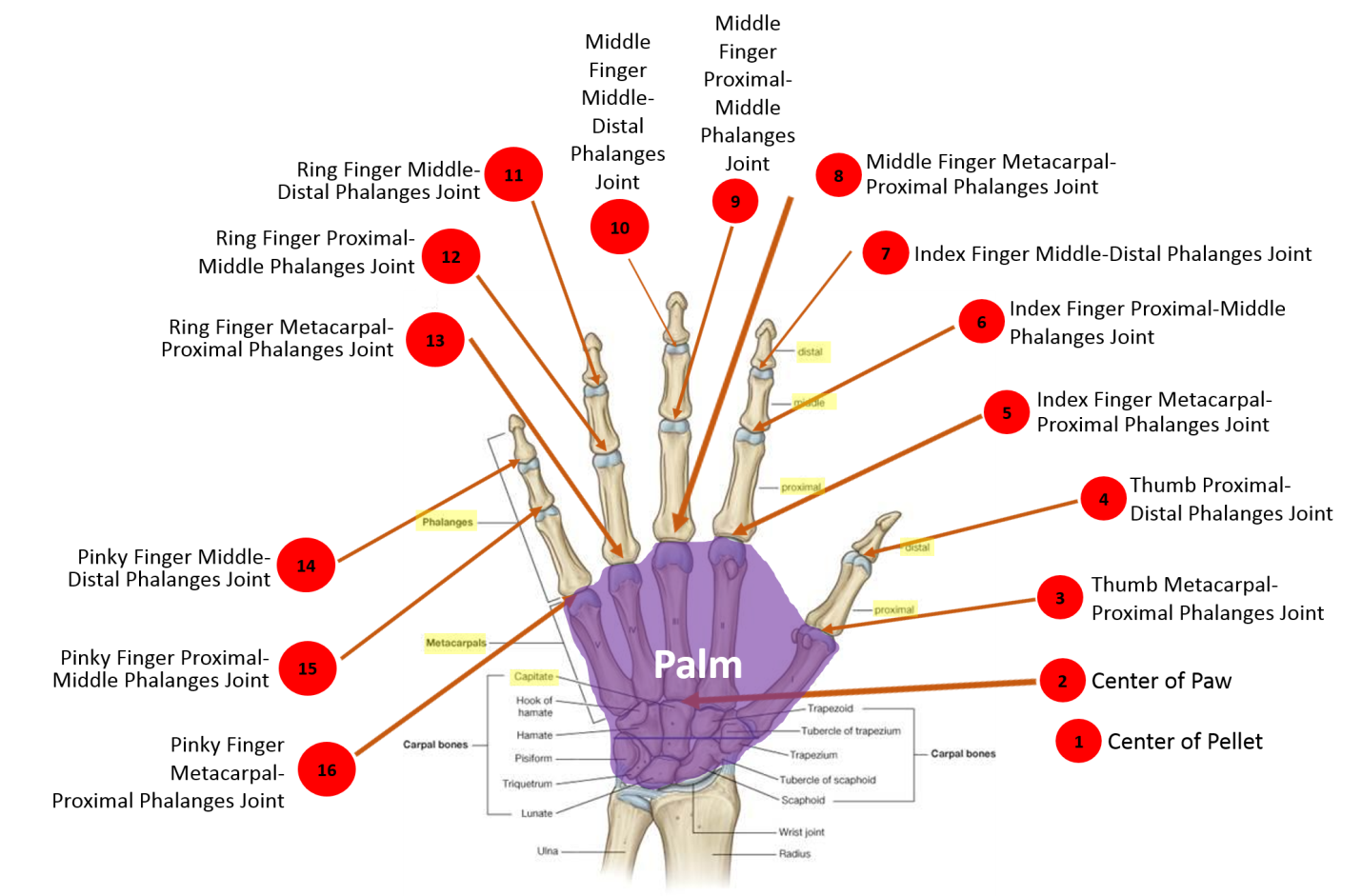


Figure 4 Marker placement diagram

* + 1. If you make a mistake while marking, you can press Delete or Backspace to re-do *that* marker. If you realize you’ve made a mistake after submitting a marker, you can click the marker(s—hold Ctrl while clicking to choose several) and hit the Redo button to redo the markers, following the prompts as indicated (again, MATLAB will display important information about the marker in the Command Window). If you cannot see a marker, press Enter instead of clicking anywhere and it will skip to the next marker. All of the important information will be saved into the RatData structure, as stated in the previous table.
       1. The Redo button unfortunately creates an inconsequential error once you’ve completed all the markers you selected to redo (the error is displayed in the command window, you can ignore it). Just follow the prompt in the popup that appears when you finish marking and click ***only the Resume button – nowhere else*** – when you are done.
  1. Once you place a marker, a small window will show up in the bottom left corner showing where in the larger image the marker was placed. ***BE CAREFUL NOT TO CLOSE THIS WINDOW***, the rest of the program depends on it being open. Let the program open and close this window on its own. If you want to check where that marker was placed, or need to see the frame overall to place the next marker, feel free to maximize this window—just remember that you must return to the 3 image figure to place markers. Your screen should resemble Figure 5 (below). Refer to the original paw-marking GUI window that appeared (on the right side of Figure 5) to know which marker to place next.

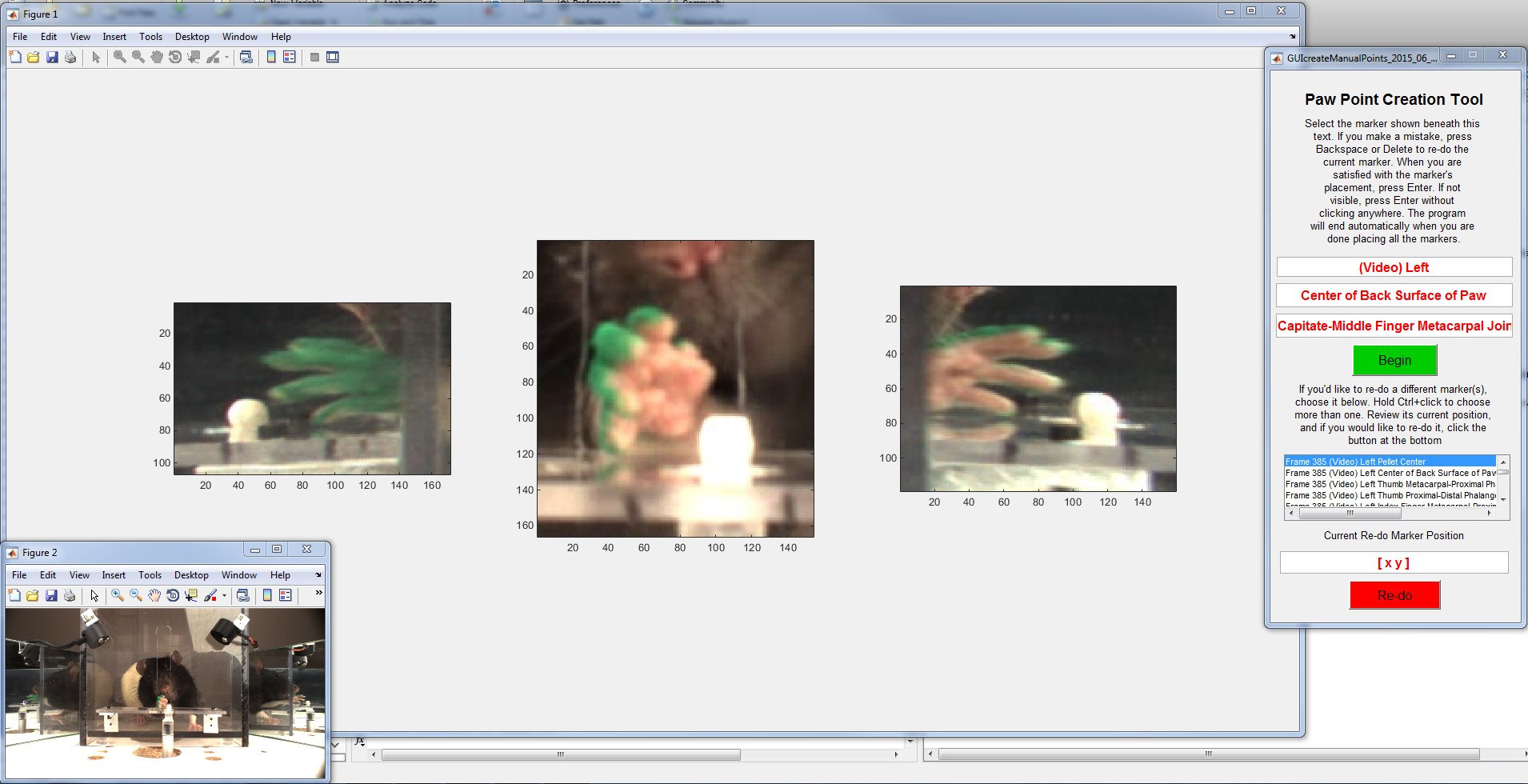


Figure What your screen should look like during marker placement

* 1. Continue placing markers, following the directions given in the paw marking GUI window. If you cannot see a marker in a given frame region, remember you can always click Enter to skip it, and you can fix incorrectly placed markers using the Delete button (if you’re trying to fix the marker you’re currently working on) or the Redo button on the GUI (if you’re fixing a previously placed marker). When you’re done marking points for a given frame, the program will automatically move to the next frame and ask you to place the rectangles, etc. again.
  2. Once you’re done marking all the frames, the paw marking GUI window will close automatically, move on to the next video/trial in the session/date folder. Once you’ve completed all the trials/videos in a given session/date folder, the program will end and take you back to MATLAB.

1. If you would like to examine the data collected so far, navigate to the rat’s processed data folder in NAS4 (ex. For RatID R0029, the filepath on a PC would be \\172.20.138.143\RecordingsLeventhal04\SkilledReaching\R0029\R0029-processed). There you should find a .mat file titled [RatID]PawPointFiles.mat (e.g. R0029PawPointFiles.mat). Once you’ve verified this, open this folder in MATLAB by copying the filepath into the folder navigation line (see Figure 6) and hit Enter.

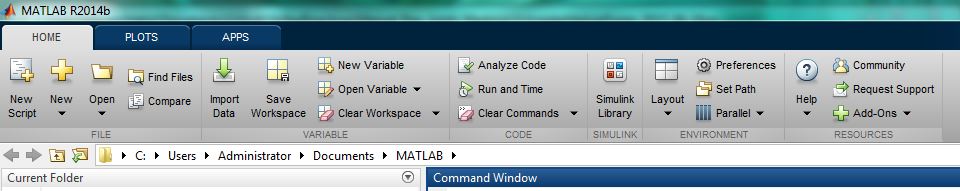


Figure 6 Folder Navigation Line (highlighted)

* 1. In the Current Folder tab in MATLAB, find the file from before and double click it. MATLAB should display “>> load('R0029PawPointFiles.mat')” in the command window, and after a brief delay, you should see something like the following in the Workspace tab:

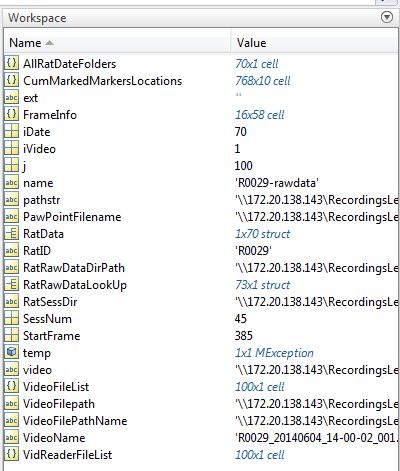


Figure 7 The contents of R0029PawPointFiles.mat

* 1. Using Figure 1 at the beginning of this document, you can explore the data created. Most importantly, the pixel coordinate data for the various markers is contained in RatData 🡪 VideoFiles 🡪 Paw\_Points\_Tracking\_Data 🡪 Columns 7 (x-coordinate in pixels) & 8 (y-coordinate in pixels). Here you can find which was the last marker placed (i.e. the last row with either a number or ‘NaN’ in Columns 7 and 8), its frame number (Column 2), the region of the frame it is in (Column 3), and the name of the marker (Column 5 for the finger information, and Column 6 for the anatomical information), among other data described in detail in Figure 1.