## **Open Field Tutorial**

- 1) Run MATLAB and change working directory to autotyping/OF.
- 2) Type OFGUI in the command window to start the open field GUI. You should get the following.

OFGUI		
	Step 1	Video description
Select a folder of open field videos		
OR		Mouse Tag
Load info mat file		
		Start time (min:sec) Frame #
Current Directory		Video duration
		(mm)
		Arena dimensions x
05164		(default 12x15)
OF VIDEOS		Perimeter (default 2)
		Apply
	Step 2	- Fix background
		Show background Eyedropper (fix background)
	Step 3	- ROI selection
		Arena surface
		Show ROIs
	Char 4	
-	Step 4	✓ View segmentation
	(optional)	
(0) Estimate Backround (batch)		Threshold =
		Save & Batch OR Process this video

- 3) Start by selecting a folder of open field videos. Each video name should appear on the left hand side in the listbox.
- 4) Once all the videos are loaded, click the "Estimate Background (batch)" button that looks like the one below. It is found under the list of video names



- 5) As MATLAB is batching the background, it will appear that the GUI is frozen. It will say on the main screen which video it is batching. Once it is complete you will be able to use the GUI interface again and the push buttons in step 2 and step 3 will no longer be grayed out.
- 6) The next step is to fill out the video information like shown below.

Step 1	Video description	
	Mouse Tag	2783
	Start time (min:sec)	0:33 Frame #
	Video duration (min)	10
	Arena dimensions (default 12x15)	16 × 16
	Perimeter (default 2)	
		Apply

- 7) Mouse Tag is the mouse number.
- 8) Start time is after the mouse is loaded into the arena and the person's hand leaves the frame.
- 9) The video duration is usually 10 (for ten minutes)
- 10) The arena dimensions are 16x16 (for the normal open field arena, in inches)
- 11) The perimeter is 2 (for 2 inches from the border of the maze is considered inner region).
- 12) Once all the video description information has been entered, hit the "Apply" button. It will take a moment to determine the frame number for the start time specified.
- 13) Once that is finished, check the background using the "Show Background" button and make sure that it looks good.

Step 2	Fix background	
	Show background Eyedropper (fix background	)
	I	

## 14) It should look something like:



- 15) If there is something that needs to be fixed, use the "Eyedropper" button.
- 16) This allows you to draw on an area where the background is correct, and then paste that region over the area that has problems.
- 17) The next step is to draw the ROIs (regions of interest)
- 18) Click the "Arena surface" button



19) Draw a border around the surface of the arena, connecting the points in each corner. It should look like the green square below. You can check it by clicking the "Show ROIs"

button



20) Click on View Segmentation to see the segmentation of a randomly selected video frame. Segmentation is done by thresholding background subtracted image. If you do not use the slidebar to set a threshold value, the algorithm will automatically choose the optimum threshold for each frame. If you select a threshold (say 80), that threshold will be applied to all frames in the video.

Step 4 (optional)		Þ	View segmentation
	Threshold =		

- 21)At this point, you may click on "Process this video" to visualize the tracking. Since each video frame is being displayed to the user, it will take a long time to process the entire video. You may click Ctrl+C in the command video to stop processing once you are satisfied with the algorithm. If not, adjust the threshold for better segmentation.
- 22) Move on to the next video and repeat.
- 23)Once done, click on "Save & Batch". This will save all work to an info.mat file. You have the option of batch processing the videos now or later. If you choose to process them later, exit the GUI.
- 24)To process the files from command line, type "edit Read\_Fnames" into the command window. (Once again capitalization is important)

25)A screen should pop up like the one below with lines of green text. If any lines are purple, put a % in front of them. This should turn them green.

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- 26)Go to the folder where the videos were and once again copy the pathway (it will be pasted into the Read\_Fnames screen).
- 27) The file name for each folder that you want to run will need to be copied and pasted
- 28)Paste the file name in the Read Fnames screen and text will be black in color. The file name should be something like 'C:\Users\admin\ Desktop\test OF'
- 29)Add a backslash (\) to the end of the file name, and apostrophes at the beginning and end of the text.
- 30)Once all of the files names have been copied over and formatted properly close out of the window and save the changes.
- 31)In the command window type: OFBatch
- 32) It should say "Busy" in the bottom left corner
- 33) The videos are now being analyzed and there will be a OF\_results folder made within each parent folder.
- 34)There will be a summary figure for each video processed, showing the trajectory of the animal and a heat-map of its location throughout the experiment.
- 35)A results.txt summary file is generated. For each file, it lists the mouse tag #, total ambulation (in inches or whatever units you specified in the GUI), total time spent in outer and inner regions of the maze in 10 minute intervals. For example, outer1 = time in the outer region in the first 10 minute interval, outer2 = time during second 10 minute interval, etc. Thigmotaxis is the fractional preference for being in the outer region of the maze.