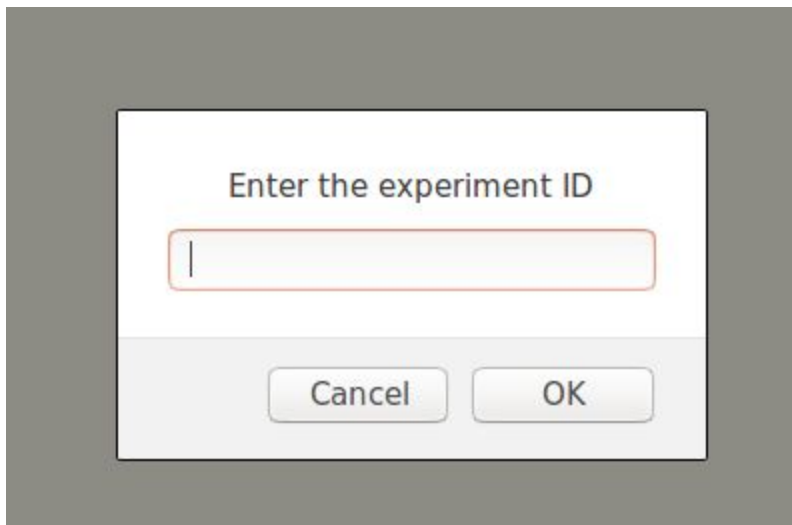


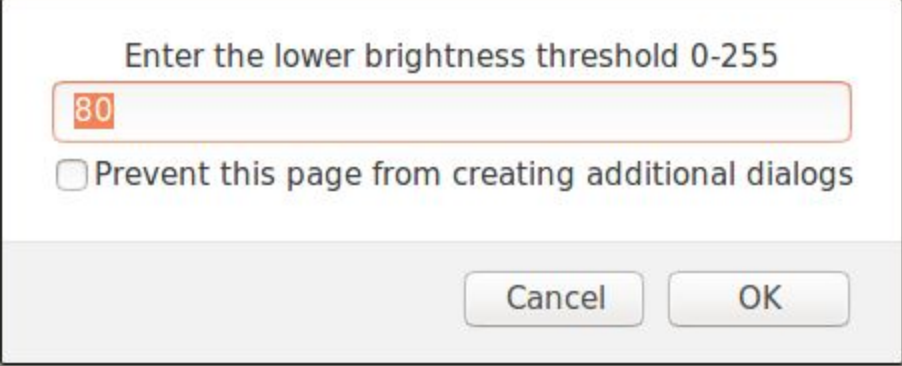
Scoring an experiment using the Retrograde application

Requirements: retrograde requires a javascript enabled web browser and a 3 button mouse to be used.

1. To access the Retrograde application on your WormBot open a web browser and navigate to <http://127.0.0.1/retrograde/retrograde.html>
2. A popup dialog will open to ask you for the experiment ID you wish to analyze. Enter the number and click OK



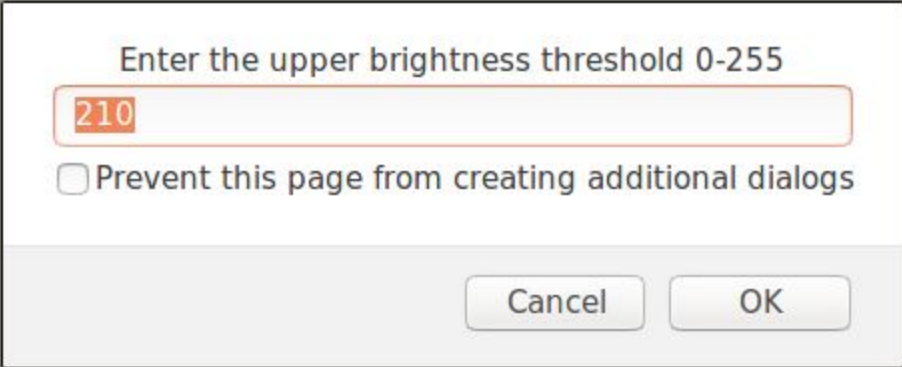
3. Two more pop ups will appear to set the upper and lower 8-bit pixel value thresholds for the edge finding algorithm the Retrograde software uses to identify the worms. Enter a number between 0 - 255 or just click OK to accept the defaults.



Enter the lower brightness threshold 0-255

Prevent this page from creating additional dialogs

Cancel OK

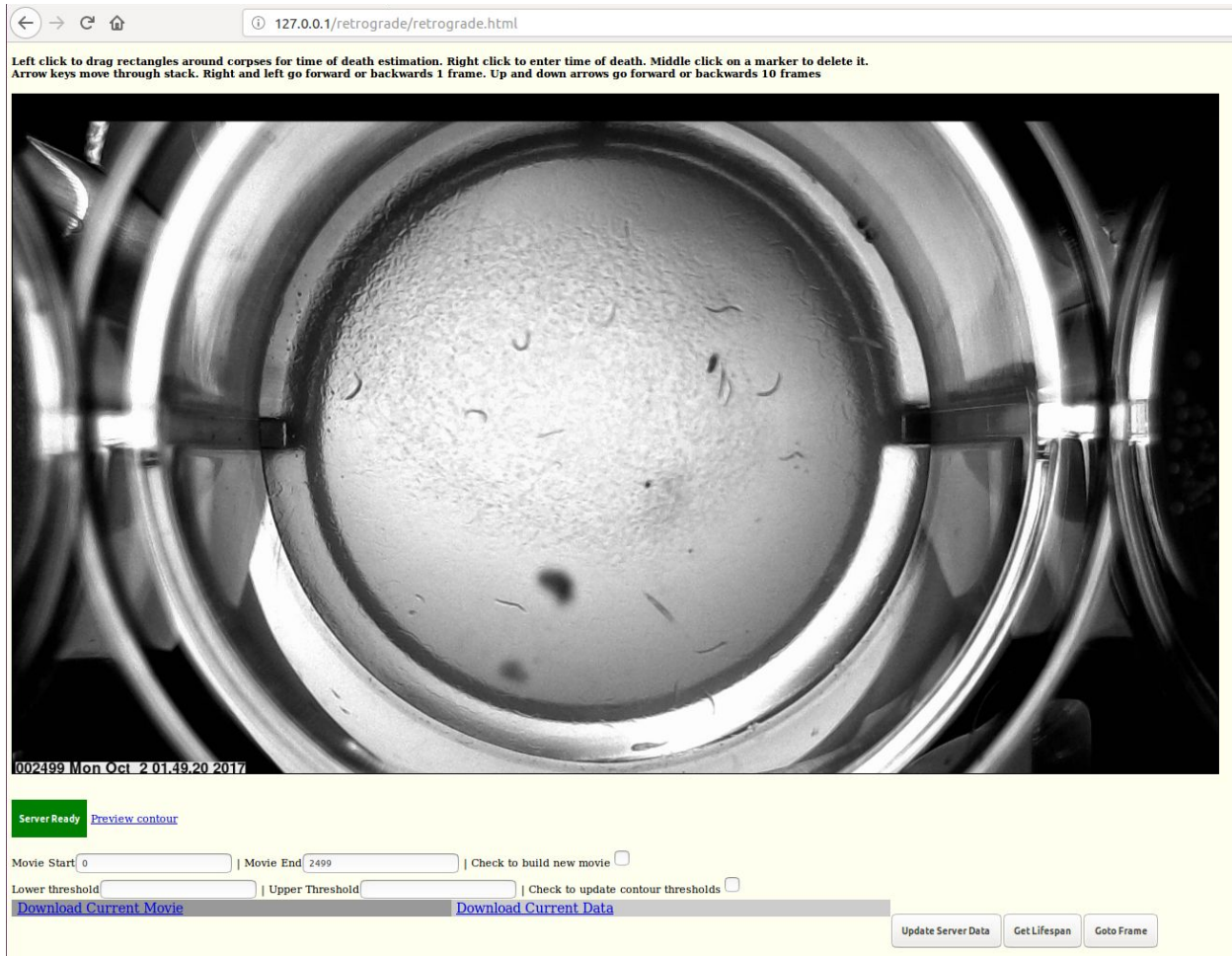


Enter the upper brightness threshold 0-255

Prevent this page from creating additional dialogs

Cancel OK

4. Upon loading the Retrograde software should display the last (most recent) image of the dataset along with the interface. If you can't see the entire interface you may need to zoom out your web browser window (in Firefox the command is Ctrl + or Ctrl - to zoom in or out).

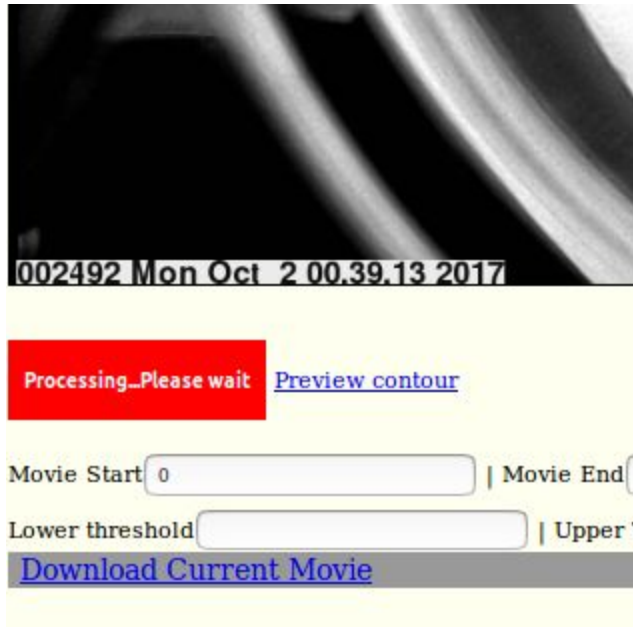


5. To work properly with the Retrograde software the dataset should have at least a day of data collected after all of the worms have died. You can use the keyboard arrow keys to navigate forward and backward in the data set to make sure that all the worms are dead before proceeding with the next step. The left and right arrow keys move backwards and forwards in time by 1 frame, while the up and down arrow keys move forwards and backwards by 10 frames.

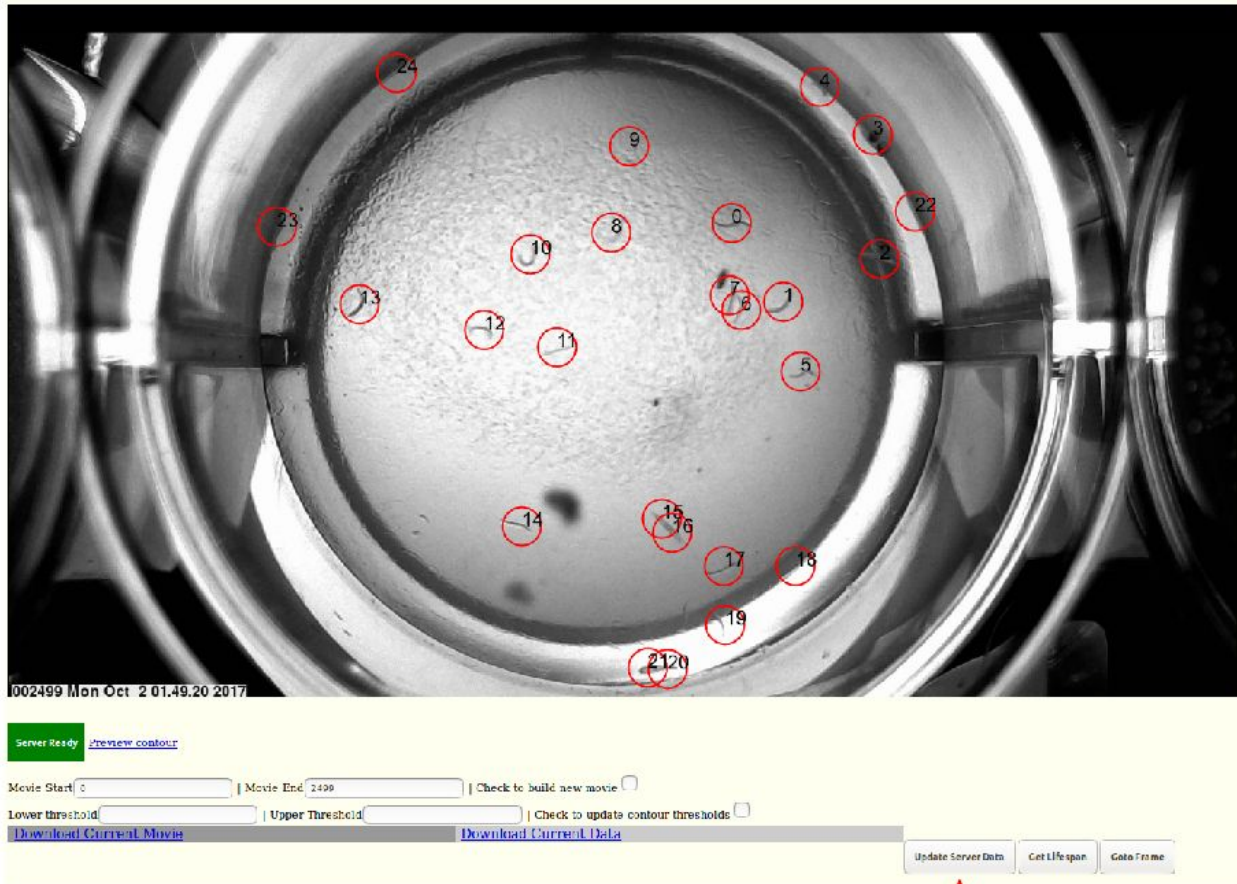
6. When you reach a point where all the worms are dead. Use the left mouse button to draw green boxes around each visible corpse in the dataset. If you make a mistake you can middle click inside a box to delete it. If worms died very early in the data set and corpses decayed you can move earlier in the dataset to draw boxes for those animals while their corpses are still visible. Once all the corpses are marked click the **Get Lifespan** button



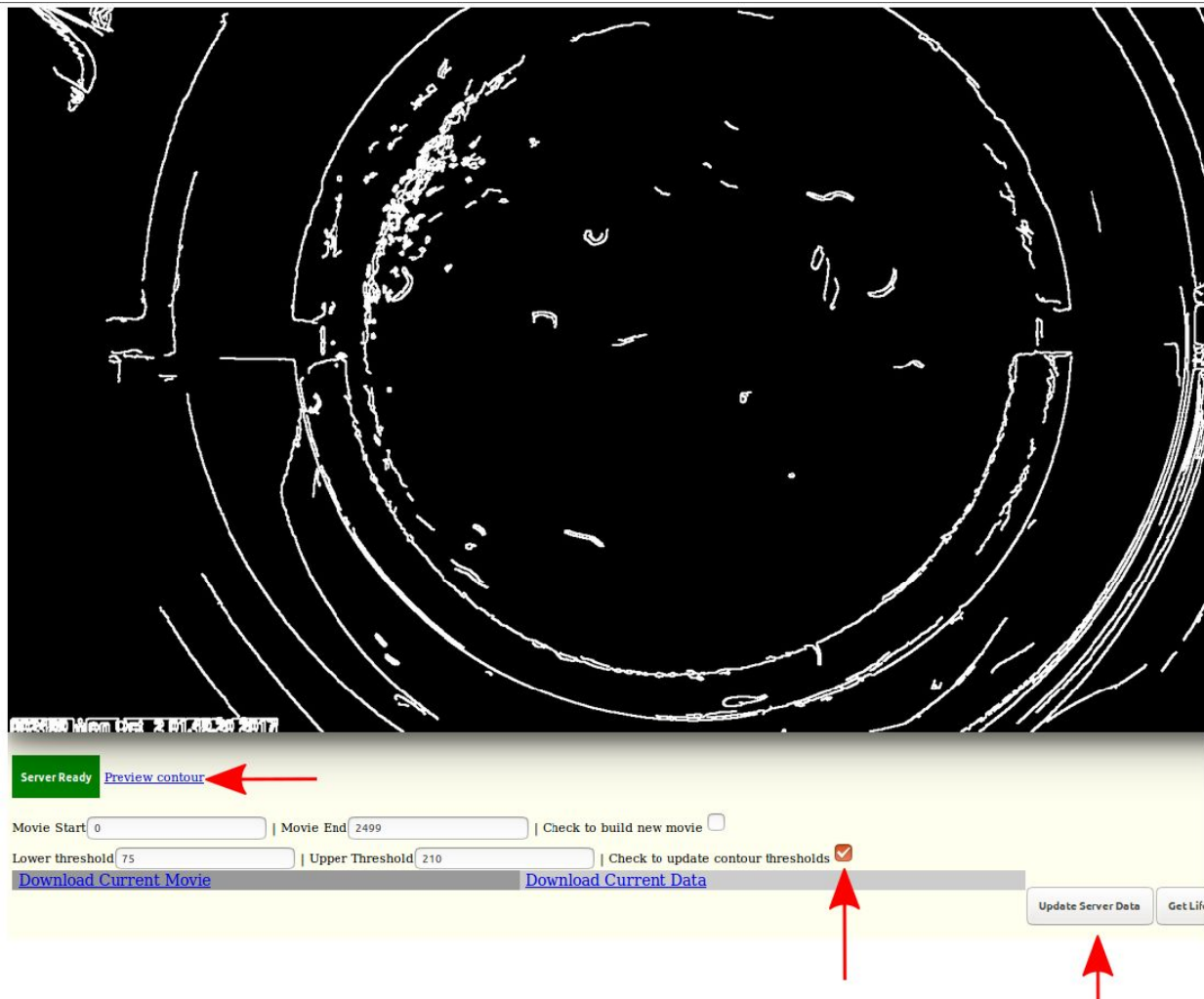
7. Once you've clicked Get Lifespan the green server ready button will turn red and read Processing..Please wait. Because Retrograde is computationally expensive and the server needs to continue to collect WormBot data during the analysis, only one Retrograde process is allowed at a time on the server. When the program is completed the button will turn green again. For an average data set with ~30 worms it should take the server approximately 3 minutes to finish.



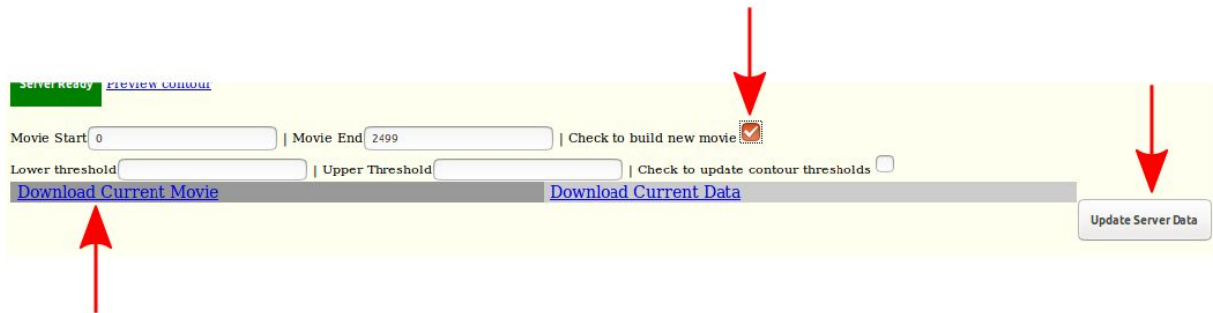
8. Checking the lifespan data. Once the program has finished running you will have the Green squares replaced by red circles marking the death times the software established. You can scroll through the data set to make certain these look ok. If you wish to modify a death time you can delete a red circle by middle clicking inside it. You can also manually enter a death time by right clicking on the corpse on the frame you wish to set for the death time much like the Marker application. After modifying the data you must hit the **Update Server Data** button to transfer the data from the Java client in the browser to the WormBot server. **Note: data is shared between the Retrograde and Marker applications**



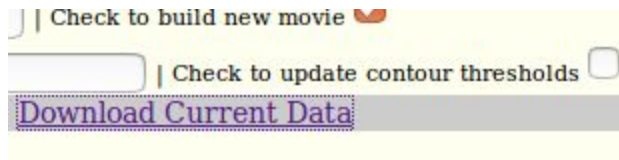
9. Adjusting Contours. Retrograde uses a Canny Edge finding algorithm to “see” the worms. The algorithm has two thresholds that need to be optimized for your lighting conditions. The default settings work well for a WormBot kept in a dimly lit lab space, but may need to be adjusted for your particular camera settings/lighting. To adjust the settings enter new threshold values in the text boxes then click the Update Contour Thresholds checkbox and finally click the Update Server Data button. At this point you can now click the preview contour link to display the current processed image. If you cannot see white lines where worm corpses appear in the image you will need to adjust the threshold values until they appear in the preview image.



10. Building Movies. To build a new movie in the Retrograde package enter the desired starting frame and ending frame in the Movie Start and Movie End fields. Click the checkbox labeled “Check to build new movie” and finally click the Update server data button. It will take a few minutes to compile the movie, after which you can download it by clicking on the Download Current Movie link.



11. Accessing the lifespan table. To get the lifespan data for analysis click on the “Download Current Data” link, which will download a CSV file that can be opened using spreadsheet software such as Excel or OpenOffice Calc.



	A	B	C
1	title	N2 on C07H6.5 RNAi wSLASHfudr	
2	email	jnpitt@uw.edu	
3	investigator	Je-Hyun Yoon	
4	description	N2 on C07H6.5 RNAi wSLASHfudr	
5	unix epoch start time	1505263657	
6	last experiment frame number	2499	
7	strain	n2	
8	experiment directory	/var/www/robot_data/42/	
9	starting N	30	
10	starting Age in days	6	
11	expID	42	
12	plate number	3	
13	well	C2	
14	N:29		
15	Mean: 21.2069		
16	Median:21		
17	Max: 24		
18			
19	%N2 on C07H6.5 RNAi wSLASHfudr		
20	0	0	
21	1	0	
22	2	0	
23	3	0	
24	4	0	
25	5	0	
26	6	1	
27	7	0	
28	8	0	
29	9	0	
30	10	0	
31	11	1	
32	12	0	
33	13	0	
34	14	0	
35	15	0	
36	16	0	
37	17	0	
38	18	0	
39	19	0	
40	20	3	
41	21	10	
42	22	2	
43	23	4	
44	24	8	
45			