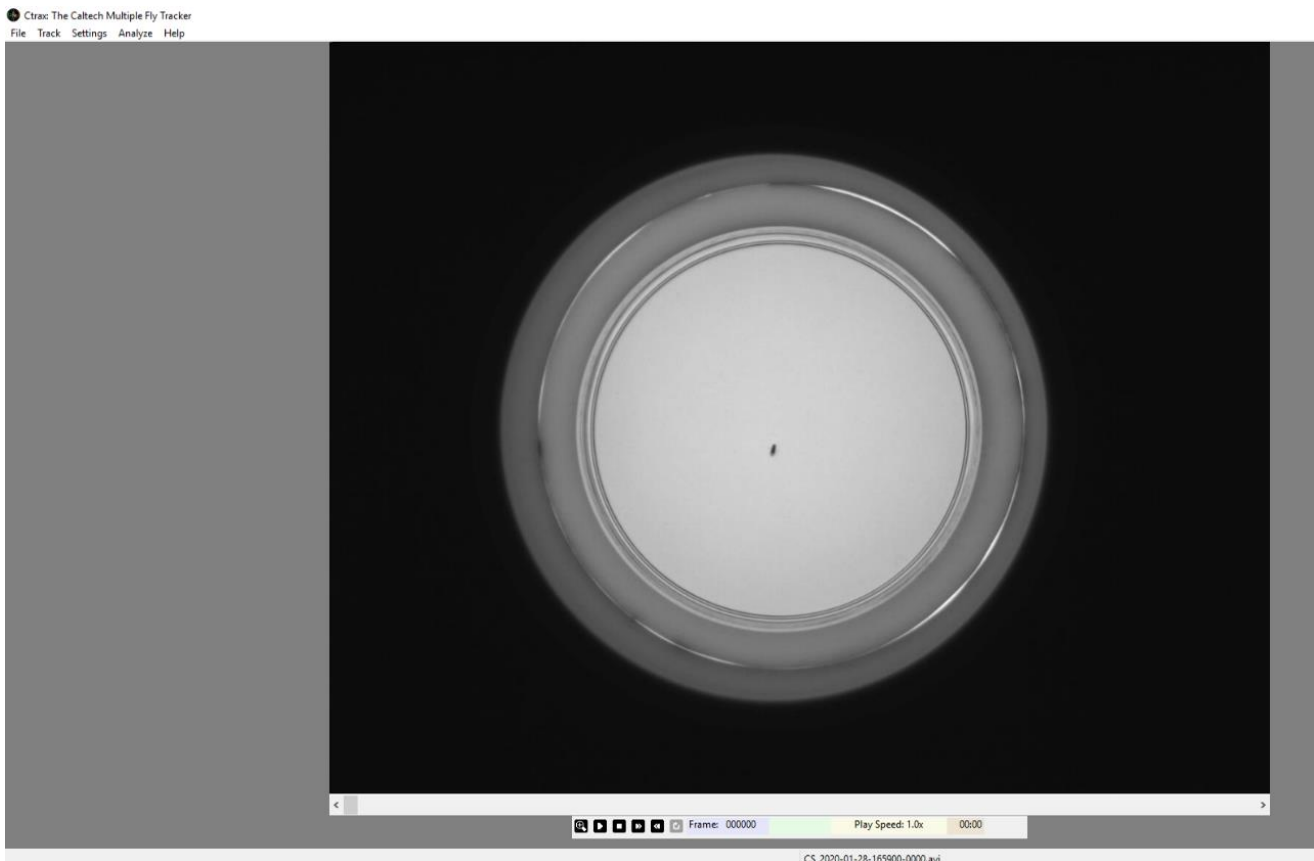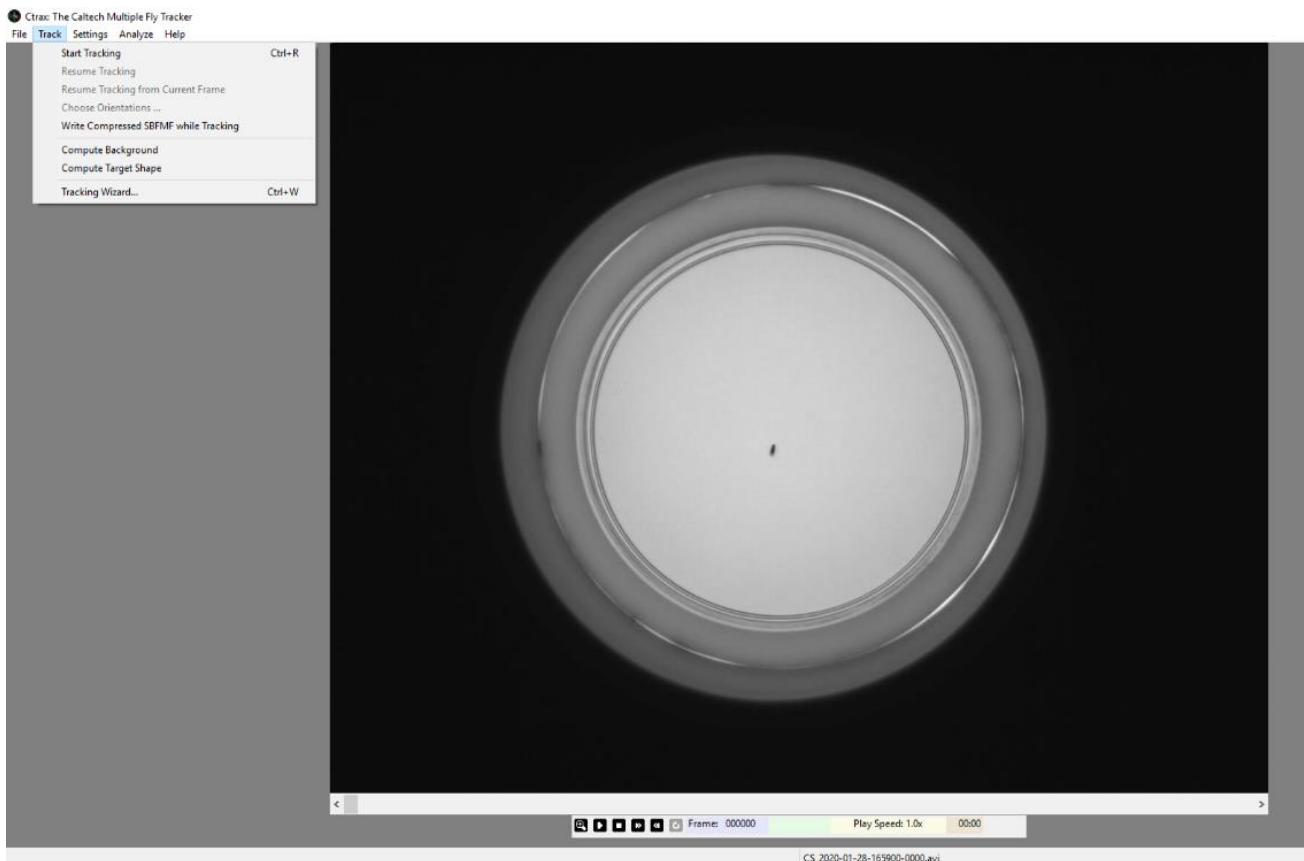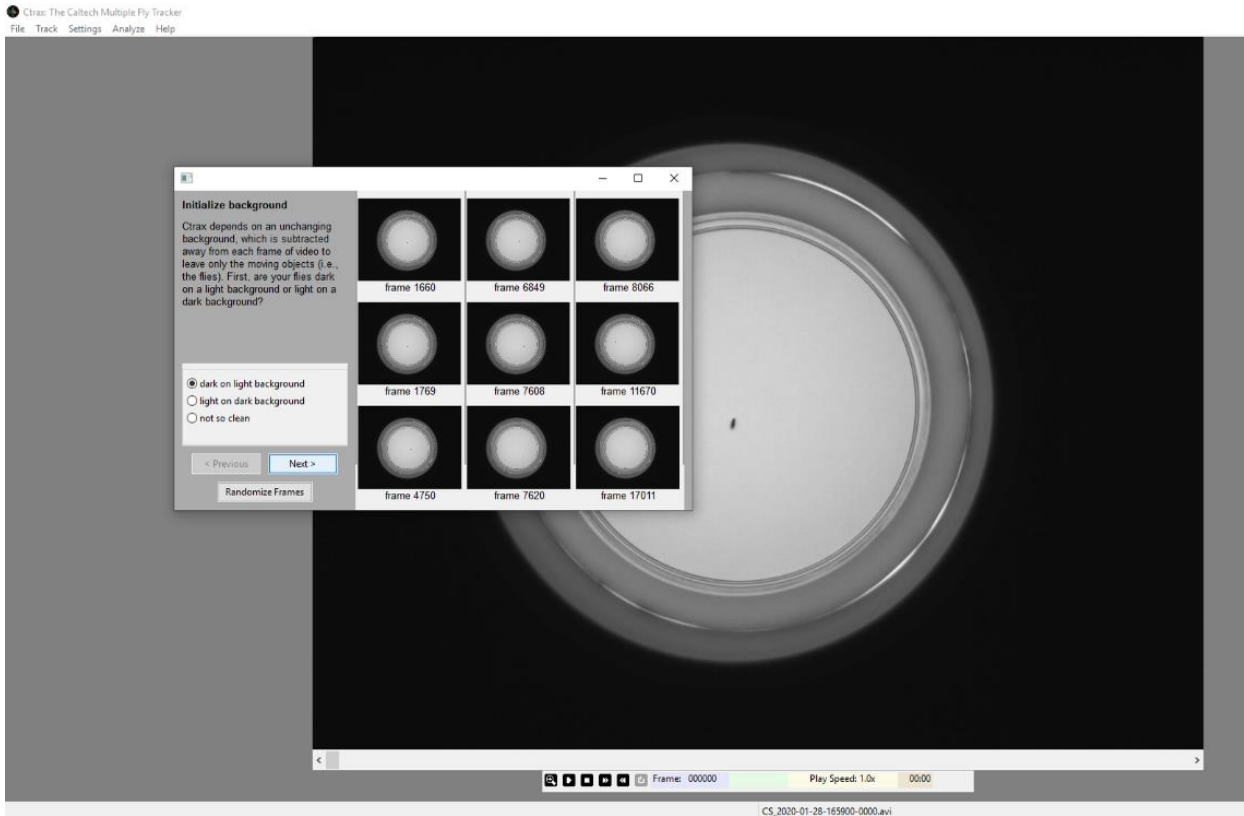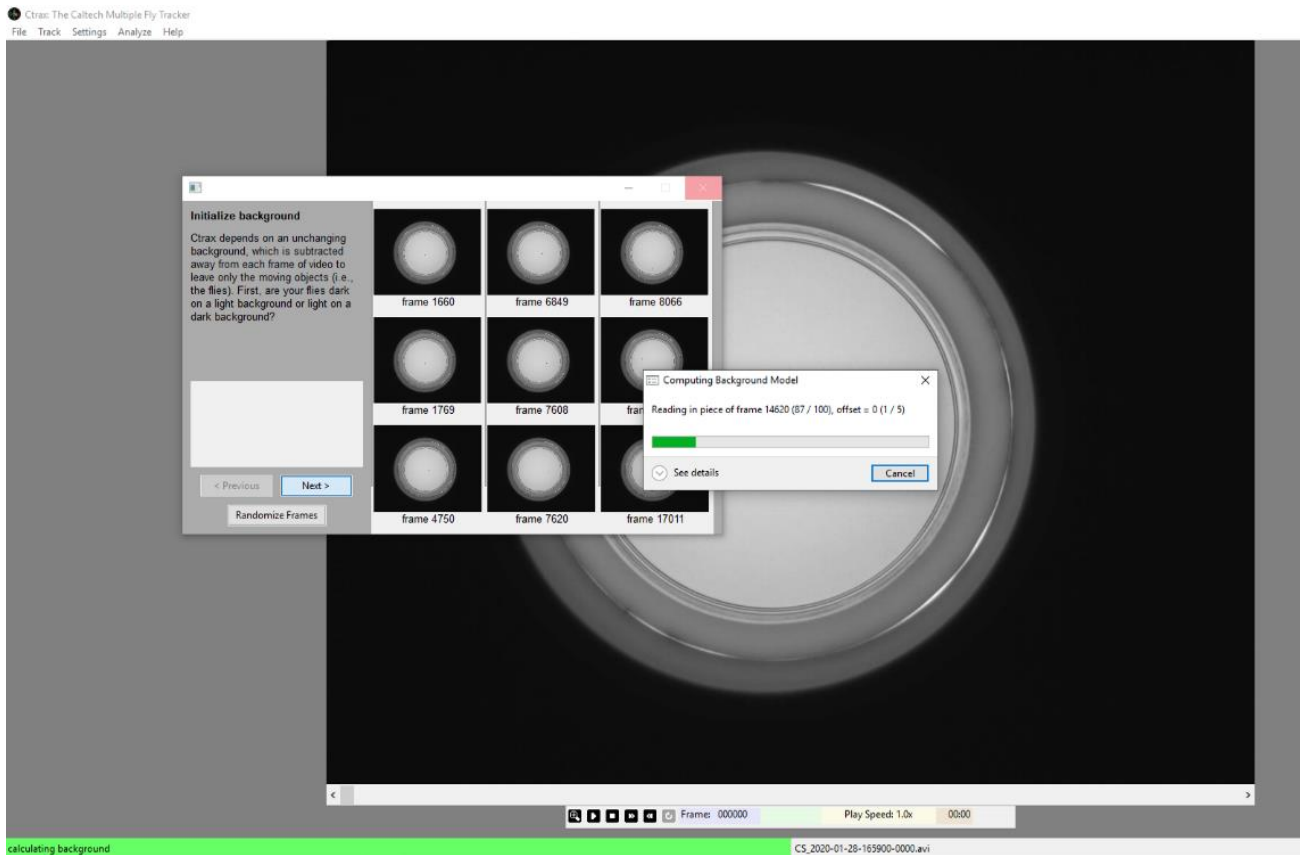1. Load the video in Ctrax.

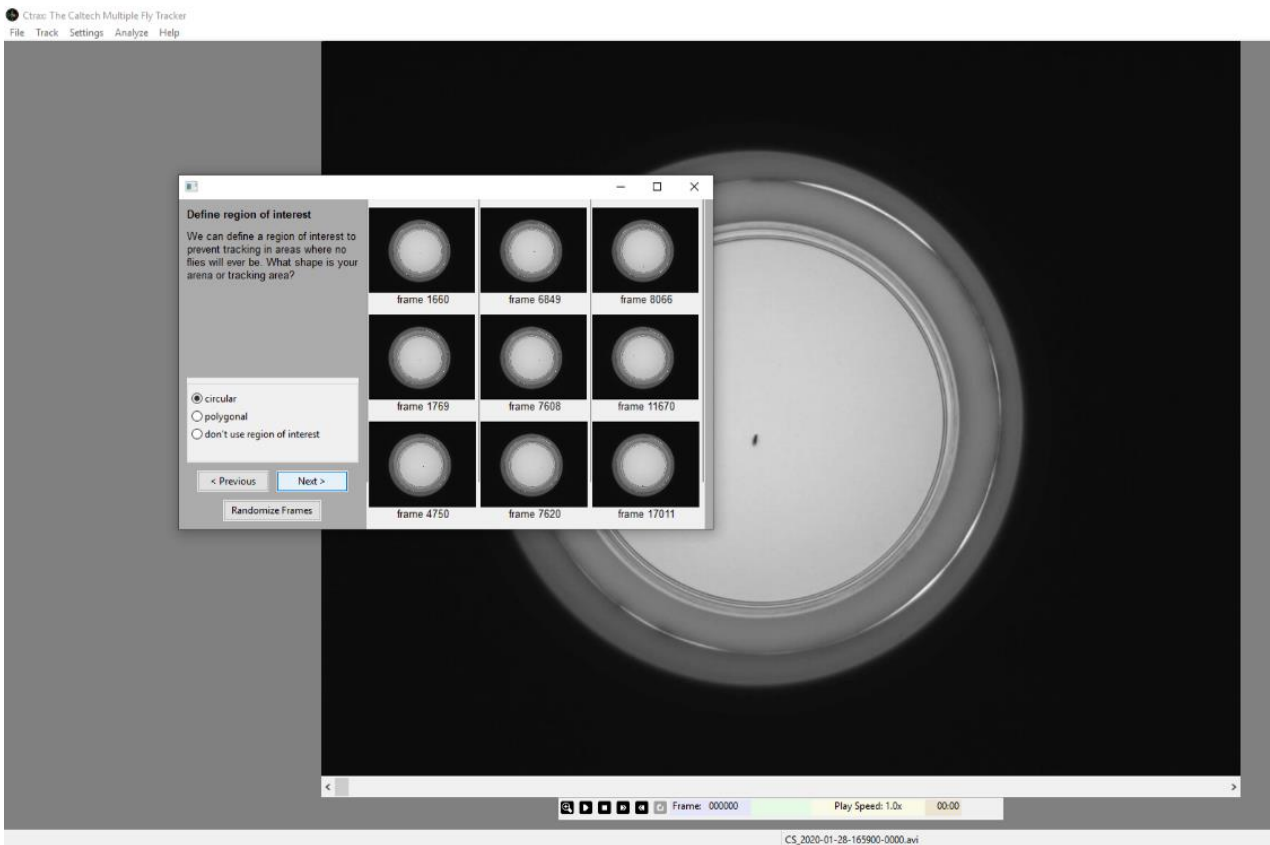2. Under 'Track', go to 'Tracking Wizard'.

3. Since Ctrax tracks moving object against a static background, select the appropriate option. Click on 'Next'.
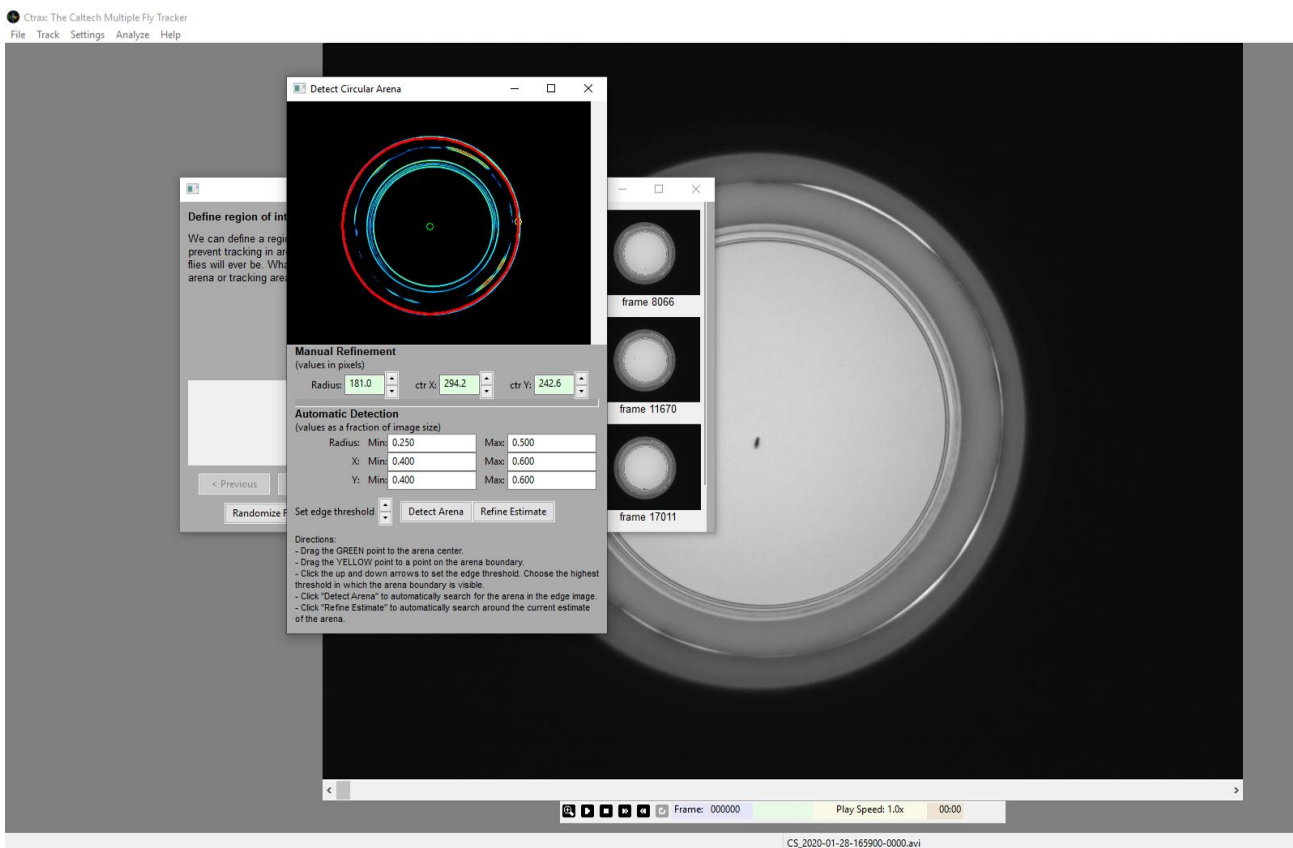

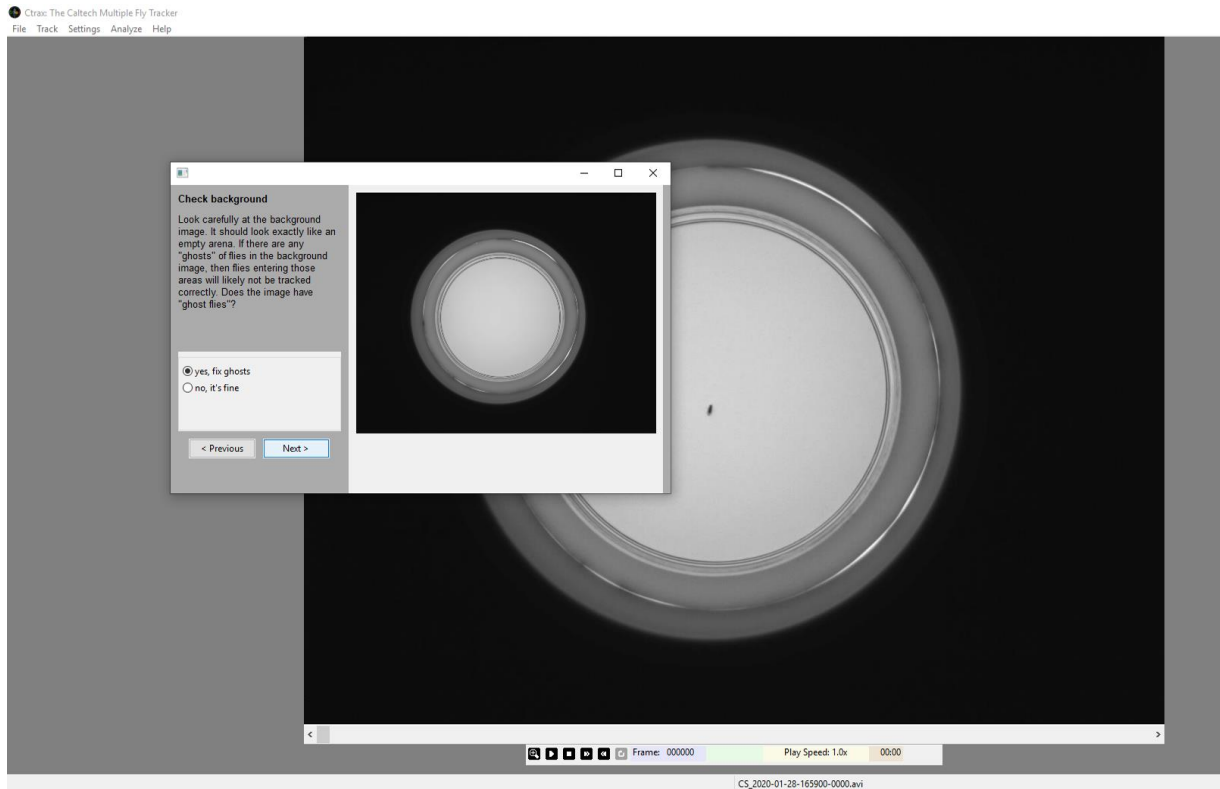4. Ctrax will then calculate the background model.
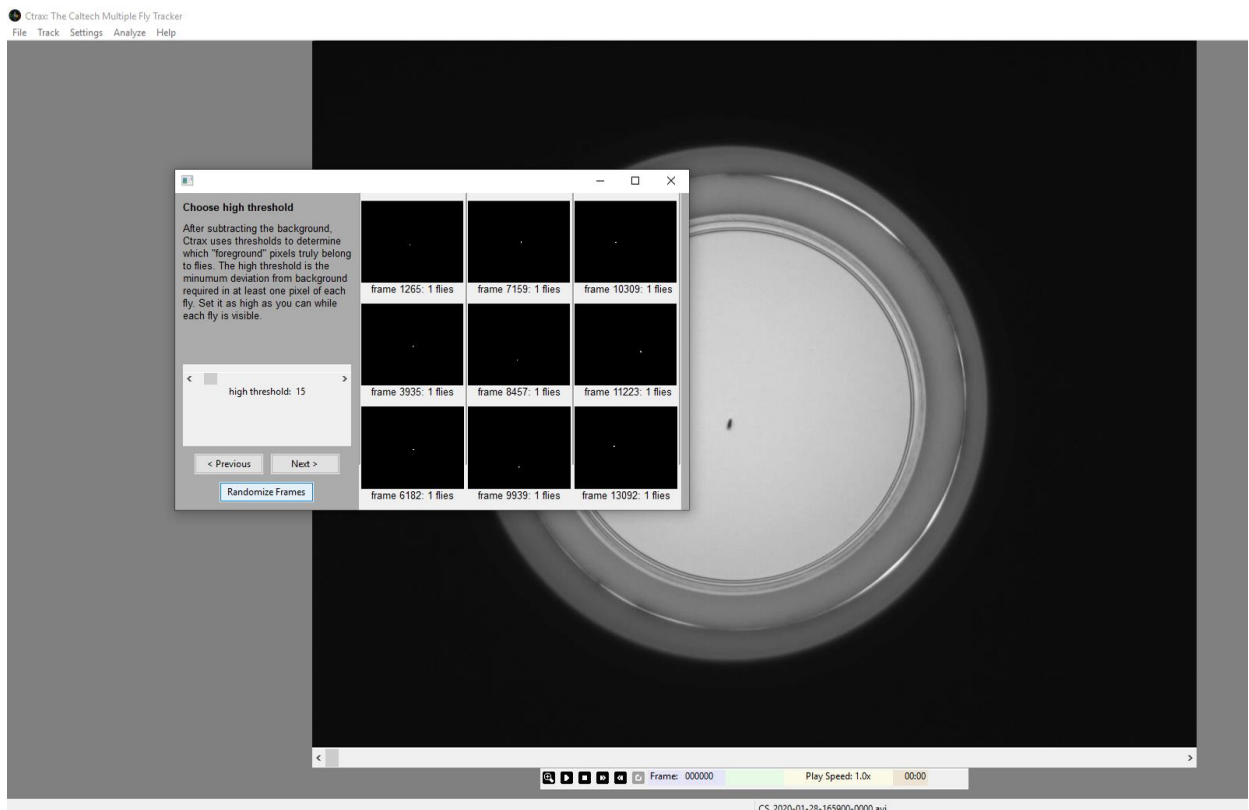
5. Select the shape of your arena and click 'Next'.

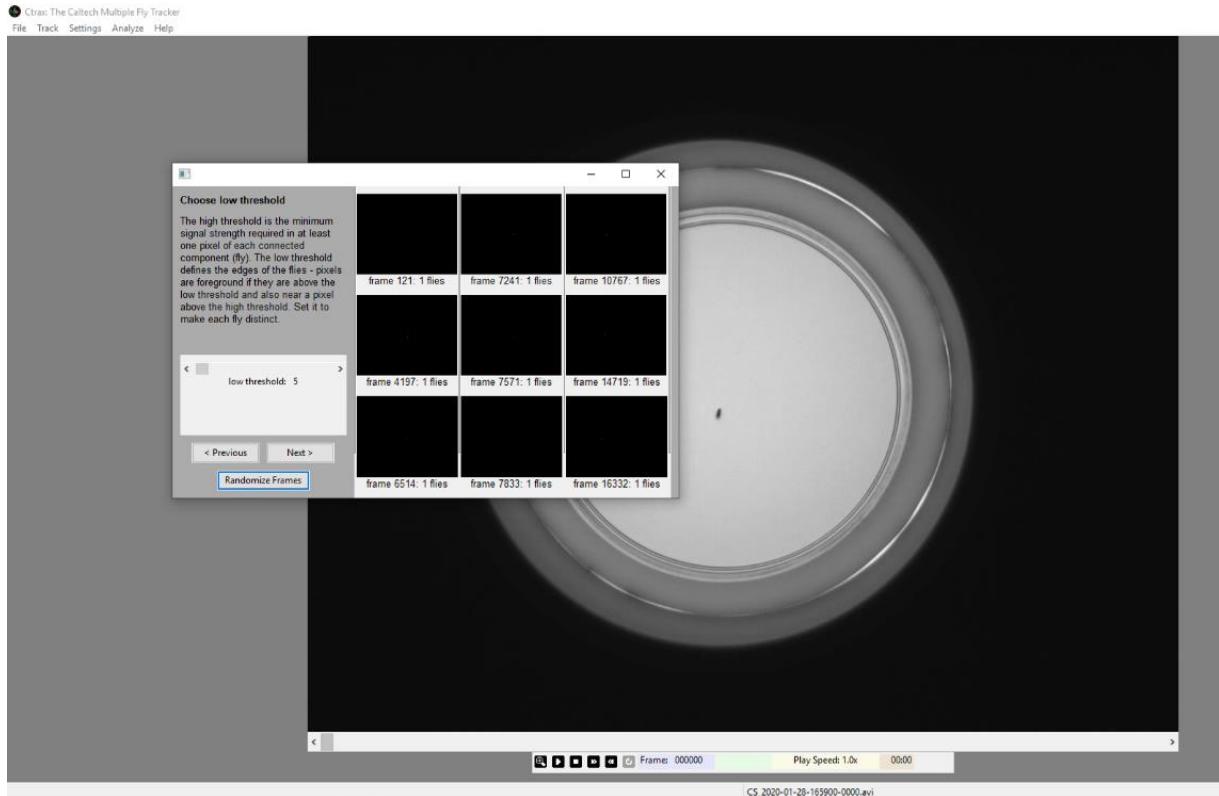6. Carefully set the boundaries of the arena. Close this window when done.

7. The arena should look empty. If you see any 'ghost flies', Ctrax will allow you to mark that area and add it to the background. Otherwise click 'no, it's fine' and 'Next'.
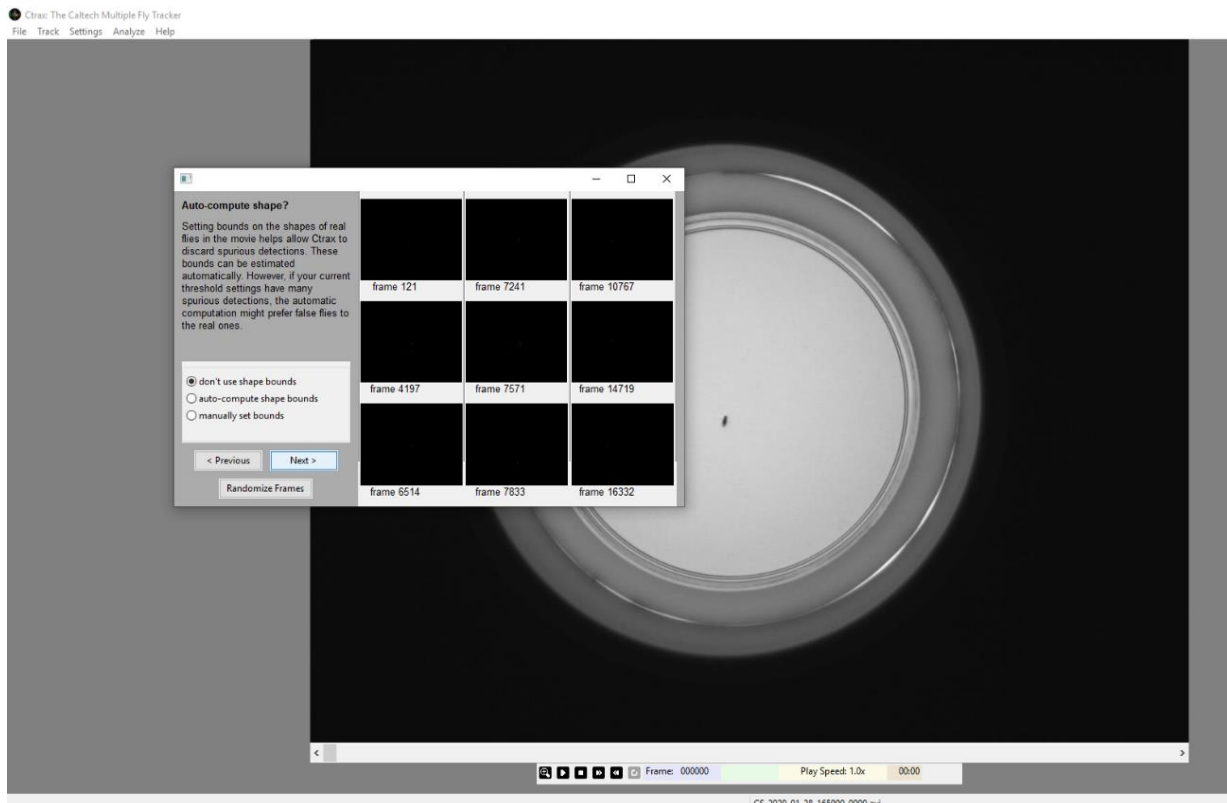

8. Now set high threshold. The default of 15 worked well for our videos. We recommend to randomize 10-15 times and check if sampled frames have only 1 fly.
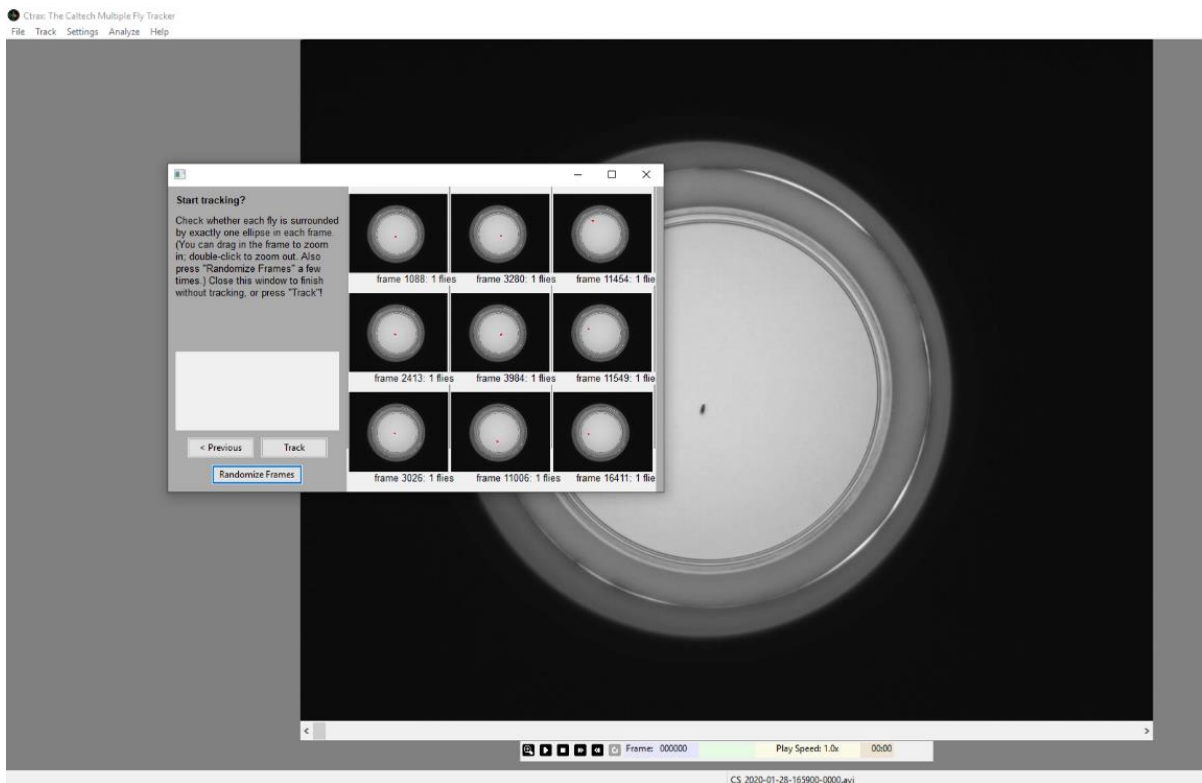
9. Now set low threshold. The default of 5 worked well for our videos. We recommend randomizing 10-15 times and check if sampled frames have only 1 fly.
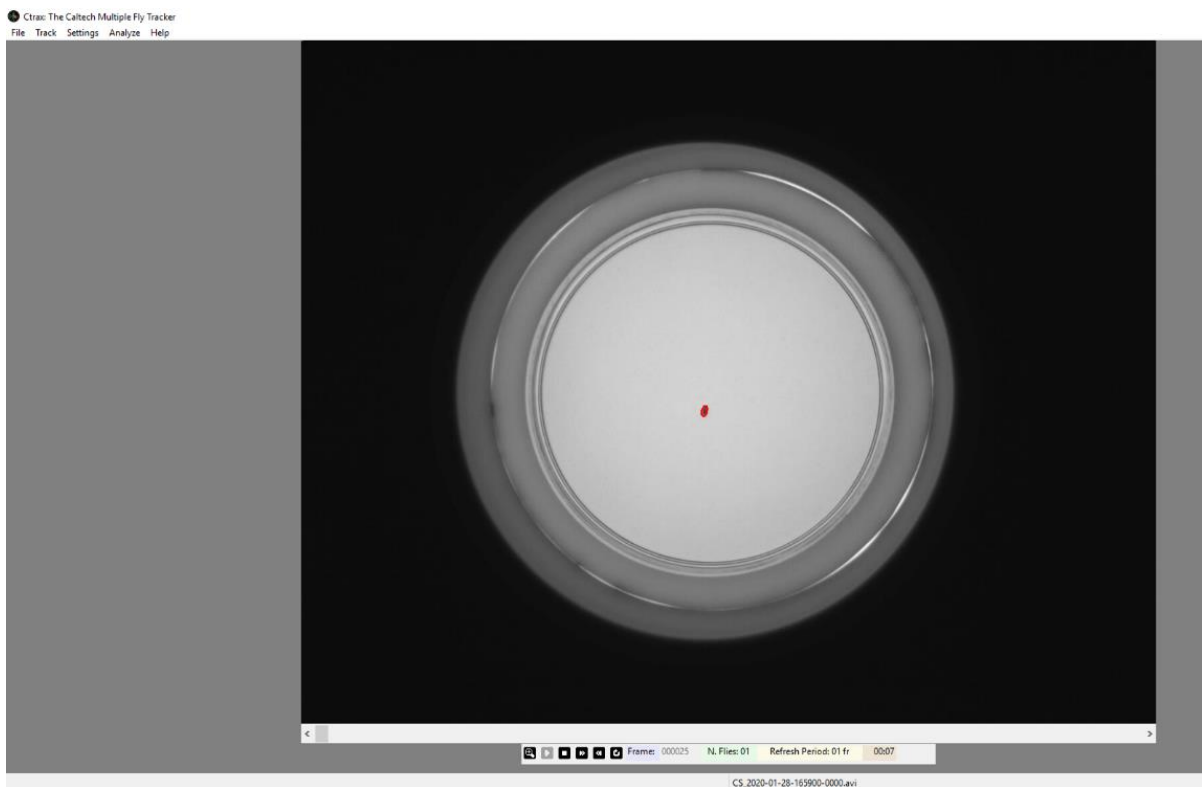

10. We did not auto-compute shape, but recommend doing so if the contrast in your recordings is not very high. Click 'Next'.

11. Finally, check if samples frames have 1 fly in all frames. Randomize 8-10 times to be
sure.

12. Click on 'Track' when done. Ctrax will start tracking.

13. When tracking is complete, (the frame number at the bottom will turn from green to purple), click on 'Export as Mat-file' and save your tracked file.