**To Prepare the Frames for Analysis, Use the “Check Right Channel” and “Creates Subfolder with Subgroup Images” Codes in Matlab. Open the codes in MatLab and press run- ensure that the labels for the frames in the code match the frames for which you are running the code. You will be prompted to identify the first and last frame for which you wish to run the code; enter the corresponding frames. Allow the code to run and save the images in the “Images Range” folder. It may take several minutes for the MatLab code to run.**

**Now that you have the specific frames with the images of the cells you wish to analyze, you are ready to open them in MetaMorph to begin tracking individual parasites. You can do this using the following protocol.**

**Protocol for Opening Movies in MetaMorph**

1. Open the” MetaMorph offline” software
2. Along the Menu at the Top, Click on “File”
3. Select the DropDown “Open Special”, then “Build Stack”
4. Select the Option “Numbered Names”
5. Go to the folder in which there are frames for the movie
6. Select the first frame of the movie you wish to analyze
7. A dialogue box will pop up indicating that you need to identify the last fame, select the last frame for the movie you want to analyze
8. The movie will now begin to load- a green bar at the bottom of the screen will move from left to right, indicating the progress of loading

**Now that the movie has loaded, you are ready to begin tracking individual parasites. To do so:**

1. Identify the cell with parasites you wish to track by watching it to see if it lyses in the duration of the movie clip; use the pencil to label it with a specific number
2. Save an image from MetaMorph of the labeled cells so as to keep track of which cells go with which trajectories.
3. Reopen the image using the “Protocol for Opening Movies in MetaMorph” above.
4. Click on the “Apps” option in the menu at the top of the page
5. Select “Track Points”
6. A dialogue box will pop up indicating that you can now track
7. Select “Add track”
8. Select a cell that does not lyse throughout the movie- track its parasites using steps 7-10 and label it as a “Control” cell
9. Use the bar at the top of the screen at which you may select frames to look at to scroll to the frame in which the lysis occurs
10. Choose a specific parasite- use the magnifying glass to zoom in. Click on the area to which you wish to zoom in. Move the bar along the side with the triangular scale down to increase the contrast between the cells and the cytoplasm- it will make tracking much easier.
11. Click on the parasite as it moves across the screen- track it for the time that you wish to track it
12. To track another parasite, click on “Add Track” again and repeat steps 4-7
13. When you have finished tracking the parasites, click on “Open Log”
14. MetaMorph will autogenerate a blank Excel file. Go back to the dialogue box in MetaMorph and select “F9”: Log Data” to automatically enter your specific data
15. A box will pop up that says “DDE” and “Text File”. Check the boxes to say yes to both options
16. The software will then prompt you to save the document in a specific folder- select the folder in which you wish to save it and click “save”
17. Go back to the dialogue box in which you tracked data in MetaMorph and select “Log Data”
18. Open the auto-generated Excel file; you data will be directly logged there

**How to Select a Parasite to Track:**

Always select parasite that can be tracked most easily and accurately. Choose a parasite that is bright enough to be able to see clearly and is not very close to other parasites with which it may be confused during tracking. Click a few frames after the lysis in order to ensure that you can see its specific movement. Do not track if:

1. The parasite disappears on the screen
2. It collides and moves with other parasites, making it difficult to determine which one it is
3. It branches off into multiple parasites, making it difficult to determine which one it is
4. The cell lyses at the very end of the movie and cannot be tracked for a significant number of frames
5. You can see the individual parasite, but it was originally in the cytoplasm and is not part of the cell that is lysing
6. There are any uncertainties other relevant about the data