Manual analysis of ultrasound images is painful. This is a well acknowledged truth, and one of the reasons so many people are keen on automating the process (another is improved objectivity). Glen Lichtwark was one of the first to come up with a solution to this problem, and during my Post-doc years, we worked together to get a working version tested and released into the wild.

When we published the <u>original paper</u> demonstrating the tracking software, Matlab needed to be open in order to run it. Nowadays, thanks largely to the efforts of <u>Dominic Farris</u> at UQ (now Exeter in the UK), you can run the software as a standalone without needing to actually install Matlab. To get the standalone version, <u>click here</u>.

Once you've got it, follow the instructions below to analyse your video:

- Open Ultratrack, then open a video file from the dropdown menu
- Define the ROI: This is your region of interest, i.e. the area you would like to track.
 The shape you draw does not matter- for an example see the demo videos uploaded with our 2011 paper in JAP
- Define the fascicle. Once the fascicle and ROI are both defined, you should see them both on the screen. Then you can start analysing
- To analyse, use the right arrow to go frame-by-frame through your video (this allows you to see the tracking result online), or click 'Process All' (in this case you only see the tracking result once it has finished analysing the whole video)
- IMPORTANT: The data will not save automatically, so use the drop-down menu to go to 'File' and then 'Save tracking data'. The saved .txt file will be in the same folder as the video you analysed

Responses to some common tracking-related questions:

*Is there a correct order for defining fascicle and ROI, or as long as both appears together and the tracking is working its fine?

I always do the ROI first, then the fascicle. Note that when you've defined the ROI it will disappear at first. Then when you've defined the fascicle they should both be visible.

*Is there any recommendation about the ROI size?

Not really. In theory, a bigger ROI is better and more reliable, but it should basically cover most of the area of the muscle you're looking at.

*Do the ROI lines have to touch the defined fascicle?

No. The ROI should ideally stay inside the upper and lower aponeurosis lines at all times so that the program is only tracking movement in the target muscle, but that is the only requirement. The program basically uses the average movement inside the ROI to help it estimate where the fascicle endpoints should be, so the fascicle ends themselves don't even necessarily need to be inside the ROI at all times. As shown in one of the supplementary files uploaded with our original paper, the tracking software is still able to estimate fascicle endpoint locations even when one or both of them are not within the field of view.

*Does the program calculate pennation angle concomitantly as well?

It does calculate pennation angle- this should appear in the 3rd column of the txt file. BUT it calculates this parameter as the angle between the fascicle and the probe, which means that in order for it to be accurate, the probe should be perfectly parallel to the muscle at all times. This is virtually never the case, so for that reason I have not used pennation angle results from this method. Of course, if you really need it, one workaround is to first track the fascicle as normal, giving you the angle of the fascicle relative to the probe. You can then repeat the tracking but this time track the aponeurosis as if it were a fascicle, giving you aponeurosis angle relative to the probe. You could then combine this angle with the 'pennation angle' from the fascicle tracking to calculate the actual pennation angle. We have done some tests with this method and it seems to work quite well.