**Ultrasound\_tracking\_v5\_0beta for semi-automated tracking of fascicle lengths during movement**

* This ultrasound tracking version was updated from v5 as it tracks tibialis anterior fascicle shortening better (i.e. less underestimation in fascicle shortening) during maximal voluntary fixed-end dorsiflexion contractions. Note that the ROI should be “fixed” because a much smaller block size is used now in the width dimension (previously it was 51, now it is 5). The MaxBidirectionalError was increased from 15 to 40 and the estimateGeometricTransform MaxDistance was increased from 25 to 100

**The following updates were also made:**

1. Before fascicle length tracking is performed, ultrasound videos that are saved as TVD files by EchoWave II software are now converted (using the ConvertTVD2ALL script and TVD2ALL function in the “updates” folder) to MP4 videos and the ultrasound frame timestamps, average frame rate, image width and height, and image resolution (cm per pixel) are stored within a TVDdata structure as a matfile. Both the MP4 video and matfile have the same filename and pathname as the TVD file and single or multiple TVD files can be converted at once. These converted files do not take up as much space compared with a matfile that also stores grayscale image data
2. The fascicle and region of interest are now outlined with dotted lines to allow the underlying structures to be seen, which were not visible before with the use of solid lines
3. An “Auto” button was added to the GUI. See below for more information about what this button does
4. An “FL\_calc” button was added to the GUI. See below for more information about what this button does
5. If you want to determine the correct image depth so that the tracked fascicle lengths displayed in the GUI are correct, you can simply load the matfile that was created during TVD conversion and then type the following into the command window: “TVDdata.cmPerPixY\*10\*TVDdata.Height”. You can then type the answer into the “Settings > Set Image Depth” input

**Auto button**

1. For this button to work, ensure the “updates” folder is added to your Matlab path
2. Once you click this button, you can then click on the superficial aponeurosis and then the deep aponeurosis, and then the ROI will be automatically defined between the superficial and deep aponeurosis. If an aponeurosis is defined incorrectly, you can debug the code to find out why, but typically it occurs because the aponeurosis is not one clear white structure and this can be fixed by increasing the input of the bwpropfilt function to >1. If you still have problems, you should either collect better images, or you could try defining the aponeurosis in a different frame of the video where it is more clearly defined
3. Following the ROI definition, a new window will be displayed that allows you to select points along a fascicle. You can change the number of points that are displayed in the code by searching for selectStrongest and changing the input. You can highlight points in the displayed window that you **don’t** want by clicking and dragging to select them and then pushing the “delete” button on your keyboard or right-clicking and clicking “Remove”. You can highlight points you **want** to use along a fascicle of interest by clicking and dragging to select them and then clicking “Confirm” at the bottom of the window. This process will fit a line through these selected points and it will linearly extrapolate this line so that it intersects with the automatically-defined aponeuroses. If you don’t agree with the fascicle that is drawn or the aponeuroses that are detected, you can simply click on the “Auto” button again to reperform steps 2-3

**FL\_calc button**

1. As fascicle endpoints drift away from the aponeurosis over time, this button can be pushed once tracking is complete to recalculate where the fascicle intersects with the superficial and deep aponeuroses. The tracking unfortunately takes much more time now because the fascicle of interest, as well as the aponeuroses are tracked across images. However, the fascicle lengths calculated from pushing this button are usually more realistic than the fascicle lengths calculated from tracking the fascicle endpoints alone