1. Using the polygon tool, draw a single complete polygon to denote the first aponeurosis:



- 2. Add it to the ROI manager (ctrl-t or edit-selection-add to manager)
- 3. Repeat this process for the deeper aponeurosis
- 4. Then from the ROI manager window, click deselect

5. Also from the ROI manager, choose *edit*, *selection*, *create mask*, to get something like this (raw image on left, mask on right):



6. Save the mask image as a .tif file (file, save as, tiff)

7. Then save the original image in a separate folder (also as .tif) with the exact same filename as the corresponding mask

- Eventually you should have a folder for the raw images, and a separate folder for the masks, with contents something like what you see below (the file names are not important, as long as they match for the image and the corresponding mask):



....etc...

## How to add fascicle labels in Fiji/ImageJ

The process is almost identical to that described above for aponeuroses. This time, you should use the straight line tool instead of the polygon tool to create the fascicle labels:

📴 (Fiji Is Just)	ImageJ											85			$\times$
File Edit Image Process Analyze Plugins Window Help															
QOG	0/	· k	*** ***	2	А	9	5mg		Dev	Stk	LUT	0	8	\$	>>
Text tool (double-click to configure)										Click	here	e to sea	rch		

Keep adding fascicles as you see fit (using *ctrl-t* as above), as long as they are clearly visible to you. **Do not** attempt to draw entire fascicles from aponeurosis to aponeurosis if they are not clearly visible:



As with the aponeuroses, keep one folder for images and one for masks, and make sure each image-mask pair have the same filename.