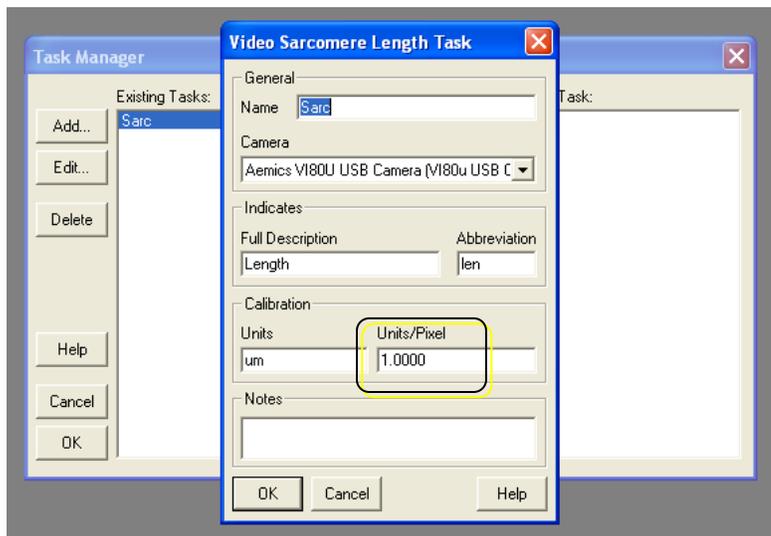


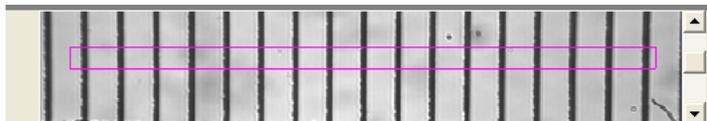
## Spatial calibration using the sarcomere measurement tool.

There are many ways to calibrate your IonOptix dimensioning system. Here is one approach for calibrating the Sarc and Edge software modules:

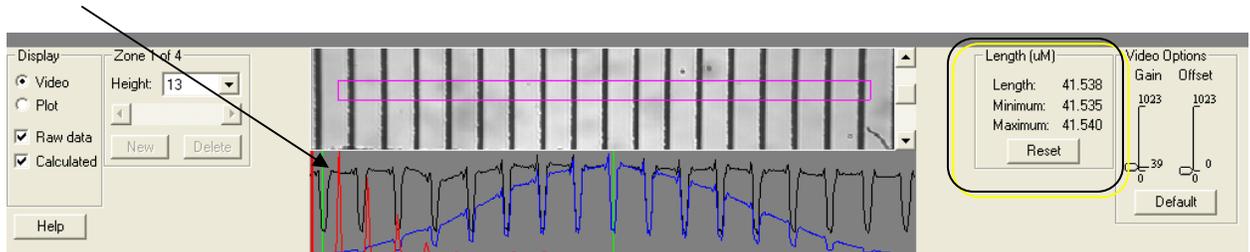
1. Go into Collect->Parameters and then Manage under Tasks.
2. Within the Task Manager (sometimes called Sources in older revisions of IW6), highlight the sarcomere recording source that you'll be calibrating (for example, "Sarc"; these are user defined so your task name may be something else). Set the Units/Pixel to 1.0. Click OK until you're back to the main window.



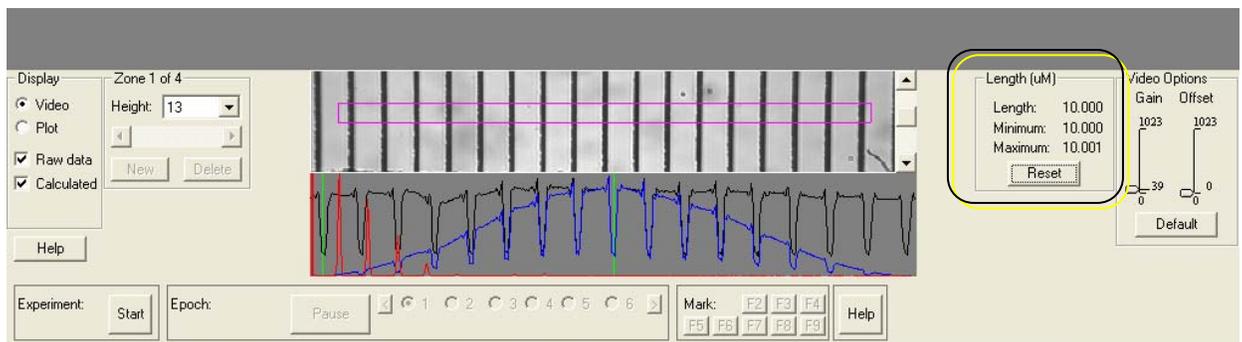
3. Mount and find the micrometer that shipped with your system. Make sure that the micrometer slide is mounted cover glass down. Visualizing the micrometer with the camera's video feed in IonWizard, set the region of interest to encompass a field of micrometer tick marks (you'll want to collect at least 10 tick marks. These tick marks are spaced 10 microns apart.



- Use the video controls to maximize the contrast. The contrast is the intensity trace, the black line in the window beneath the video. Move the lower limit in the same window to the left until you see something similar to the picture presented here. The lower limit is the green bar on the left. The power spectrum (red line) will now look like a series of diminishing peaks. The peak on the farthest left will be the one recognized by the software if it is the tallest.



- Hit the reset button on the right of the sarc acquisition window. The numbers there will now represent the average number of pixels between the gradations of the micrometer. The actual distance between them is 10 µm. So the Units/Pixel will be 10 divided by the observed number of pixels. If the max and min are 41.535 and 41.540, you could use 41.538 and get a Units/Pixel of 0.24074.
- Add this calculated value back into the Units/Pixel in the appropriate source under "Source or Task Manager". When you go back to the main experiment window, you should measure 10 microns where you measured 41.538 before.



- Because the IonWizard's edge tool uses the same hardware for measuring cell length (*i.e.*, the same objective and recording device), the same calibration factor will also apply. The edge tool typically should be used to confirm that the calibration is appropriate. If the micrometer above is used, the distance between the bars currently shown at either end of the sarc region of interest should register 160 µm using edge.