

Protocol for obtaining Speeds using CellTrak

This protocol is for use with the CellTrak system as implemented in the MacOSX environment. The dissecting scope in Mateer 309 is currently set up for capturing the necessary video. Make sure the AV converter box is attached and that you have the CellTrak dongle in the computer.

Capturing the video

- ☞ Open iMovie.
- ☞ Click on camera icon to access camera feed.
- ☞ Make sure camera box is on and begin import.
- ☞ Label imported video with the days date and experiment.
- ☞ The remaining protocol is done for filming at 12.5X magnification from the click stop knob.
- ☞ Set camera at magnification, adjust focus, and adjust brightness with microscope knobs to focus the computer screen.
- ☞ Film paramecium for 30-60s after computer image is clear.
- ☞ Stop filming and exit camera feed.

Editing the clip

- ☞ Create new project with imovie.
- ☞ Select between 10 and 20 seconds of clear video with several paramecium swimming in relatively strait paths and edit the video so only that portion remains.
- ☞ Return to project library after video is edited.
- ☞ Right click the project and export the project using Quicktime.
- ☞ Save as an .AVI file in a location you can remember.
- ☞ Exit Imovie and open VMware Fusion.

Analyzing the video in Cell Trak

- ☞ Open CellTrak from the desktop.
- ☞ From the CellTrak menu go to File->Load AVI Video Files.
- ☞ Find your movie clip in the folder system, and load it.

Is the video the right size?

- ☞ Once loaded check the amount of frames in the video in the bottom right hand corner of the screen.
 - If you video is much more than 600 frames the system can't handle it.
 - Return to imovie and edit out a few more seconds.

Calibrating the distances

- ☞ Go to the set up Tab.
- ☞ On the right side of the screen set the Calibration units to millimeters
- ☞ Set the measurements to 6.5 mm horizontal length, and 4.75 mm vertical length.
- ☞ Click "Calculate Horizontal Scale factor", and follow the on screen instructions.

- ☞ Same for “Vertical Scale Factor”.

Detecting the object (cell)

- ☞ Go to the Process image tab and set the “Edge Detection Method” to “Binary Threshold” on the right hand side of the screen.
- ☞ Fiddle with the Threshold value until you can see the *Paramecium* in the left hand picture as black dots and the rest of the picture is white.
 - Most video is good at thresholds around 175.
- ☞ Click “Apply Method to All Frames”.

Processing the data

- ☞ Go to the Metrics Tab and Click “Calculate Metrics for all Frames”.
- ☞ On the right hand side of the screen go to the “Edit Paths” tab.
- ☞ Change “stationary objects move less than x pixels” to 9.
- ☞ Click “Select all stationary objects”.
- ☞ Click “Delete Selected Paths”.
- ☞ Click Save and renumber paths, and answer “accept” to confirm.
- ☞ Click the play button in the bottom of the screen and watch the paths in the upper right corner.
 - Some non-paramecium paths may be present, simply click on the gray lines to select the paramecium paths you want.
 - Stop the video and click “delete unselected paths” to get rid of the remaining non paramecium paths
 - Save and renumber again
- ☞ Go to the Time series Tab and then click “select all paths” in the upper right, and “Mean” below that.

Analyzing your data in excel

- ☞ Toggle to the Math tab in the upper right.
- ☞ Go to File->Save Math Time Series and save you file after checking the boxes for Speed, Mean, and Standard Deviation.
- ☞ Open the save file in Notebook in the VMware Fusion and resave the file as a .txt file.
- ☞ Exit the VMware and open Excel.
- ☞ Import the .txt file into Excel and you have numerical data which you can access and change as you want.