**Measuring sperm velocity using Manual Tracking**

1. Open ImageJ and open a video file (.avi). When prompted, open it as an image stack with grey scale.
2. Open the ImageJ plugin 'Manual Tracking' under the plugins tab.
3. Set the time interval. This is seconds/frame, so you must calculate this based on the frames per second of the video.
4. Set the 'x/y calibration' to 0.307 and the 'z calibration' to 0.
5. Select the 'Show Path' box.
6. Starting at frame 1, scroll through the video to find a cell that stays in focus for at least 40 frames. We want to measure all cells in frame 1 to capture a random snap-shot of sperm cells. Click 'Add track', which will take Set the video stack to the earliest point that cell is in focus and click on the rear part of the cell's head. This should advance the image stack.
7. Manually track sperm for between 40-100 frames. At the end click 'End track'. This will allow you to move to another cell. Copy and paste the 'Results' to a new Excel sheet. Make sure to make a column on the left that has the ID for each male! Keep a paper record (drawing) of the tracks and frames sampled from each video to prevent duplicating measurements.
8. Track 15 cells for each video, preferably cells that are all in frame 1. If cells enter within the first 20 frames, those can also be measured. If there are not 15 cells to measure in frame 1, advance video to frame 100 and begin with cells the upper left corner. If frame 1 was blurry or the camera shifted in the first 80-100 frames, begin the measurements where the camera comes back into focus.
9. Copy the measurements into an excel spreadsheet. The first measurement for each track will list '-1' for both distance and velocity. Replace distance with '0' and leave velocity blank.

Motility

1. Open the cell counter, and click initialize while you are on the first slide of the video stack.
2. Choose a single cell in that first frame. Scroll forward to see if that cell is motile, if so, count it as motile, if not, count it as immotile.
3. Repeat this for 50 cells. If there are fewer than 50 cells at the beginning of the video, move forward 300 slides, and repeat. Repeat until you count 50 cells.