Measuring cell rotation by image processing and manual tracking

Videos of experiments are captured at 30 Hz for each individual oocyte, and processed manually using the free software ImageJ V1.46r manual tracking plugin, NIH, USA. In the cases where feature points are hard to determine on the cell, the video files are imported into ImageJ and each frame is further processed (via Image > Adjust > brightness/contrast> auto; Image > Adjust > Threshold > manual-threshold) to determine several key points on the circumference of the oocyte. The obtained key points are manually tracked using the Manual Tracking plug-in tool (via point-selection tool; Plug in > Manual tracking). A step by step procedure to calculate the rotation measurement is provided below:

Step 1: Import (File→ import→ image sequence) the raw video file into ImageJ as image sequences and then convert them into 8-bit files to reduce file size.

Step 2: Adjust the brightness/contrast of the import image sequences (Image→adjust→ brightness→auto).

Step 3: Set the threshold for stack file as 136 (low-level threshold) and 136 (high-level threshold) and then apply (Image→adjust→ threshold) to all the image sequences to get the binary images (like Fig. 1). Note here the threshold of 136 is selected manually for better visualization of key points and need be tailored for another cell.
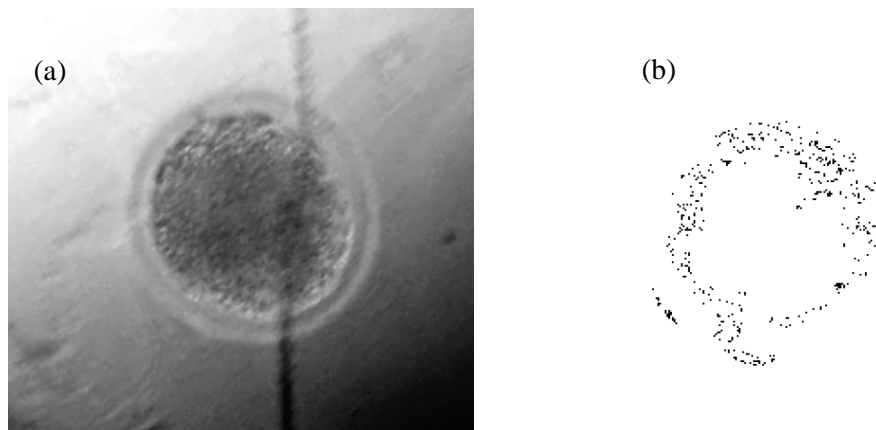
(a)

(b)

Fig 1: Image process analysis of bovine oocyte. (a) One image sequence. (b) Granulated pixel points obtained on the circumference of the oocyte after adjusting the threshold to 136 as low and high level threshold.

Step 4: Select a unique tracking feature pixel group on the circumference of the circle by mouse clicking (Fig 2). The unique pixel group is selected such that an image patch (as a template) around this group (i.e., the pixel group and its neighbouring pixel groups) matches 40-50 successive image sequences.
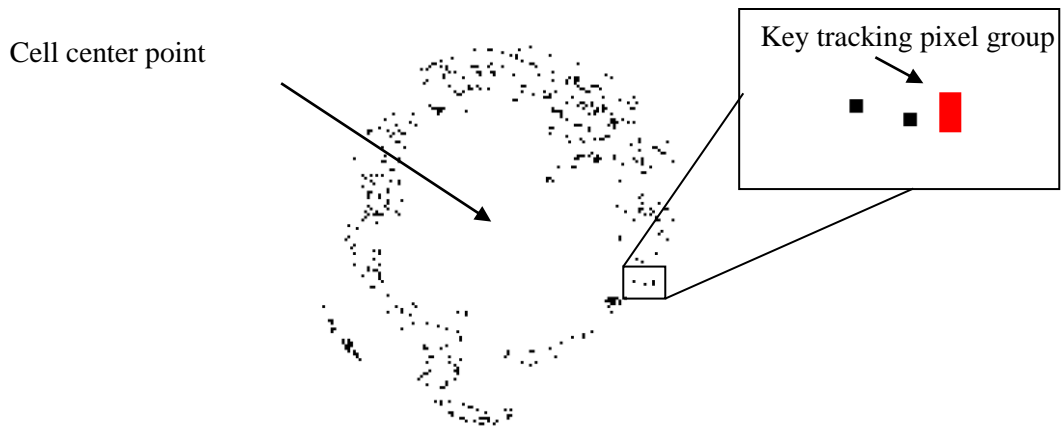
Fig 2: Zoomed in view of the thresholded oocyte image, showing selection of the key tracking pixel group.

Step 5: Manual tracking tool (Plug in→manual tracking) is used to track the movement of the key tracking pixel group in image sequences. For each image sequence, the cell center point (X0, Y0) and the key tracking pixel group position (X1, Y1) are recorded and used to calculate the slope of the line connecting them. As such, the rotated angle between two certain sequences can be easily derived. A sketch in Fig. 3 illustrates this process.
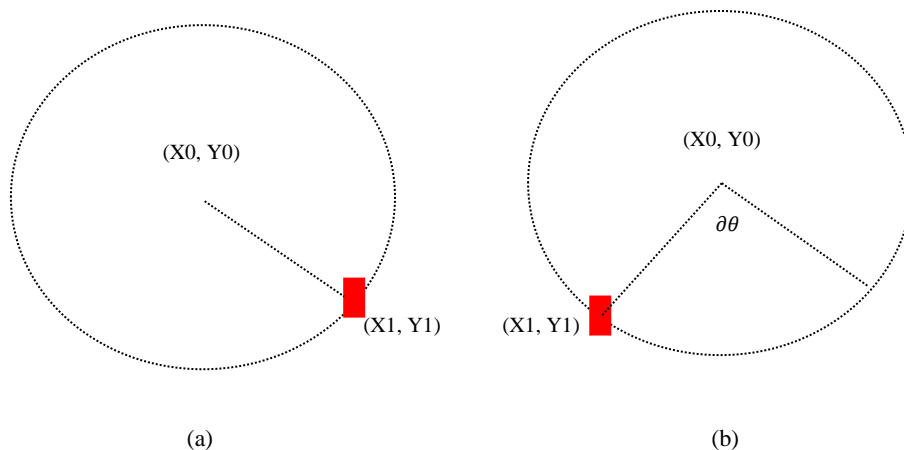

(a)   (b)

Fig 3: Manual tracking of key pixel group for rotated angle calculation. (a) The selected key pixel group centre in image sequence I0. (b) Rotated key pixel group (X1,Y1) is tracked in image sequence I0+T (T = 4-8). Slope of the lines and angle ($\partial\theta$) between the two lines can be easily calculated.

Step 6: Repeat Step 4 and 5 with a differently selected key pixel group to calculate the rotated angle three more times. Finally, an average rotated angle can be obtained and thus the rotation rate is calculated.