Reviewer 3

General Comment: I think other revisions are necessary to the manuscript. Here are my comments:

Answer: We thank the reviewer for putting in the effort to help us improve the paper. We will answer questions in green colour and make the changes to the manuscript in green colour so the reviewer can differentiate current changes (in green colour) from previous changes (red colour).

Q.1. In the water drop simulating a well of a multi-well plate? Or are the oocytes kept in the water drop during cracking and for further analysis? If the drop is only an example of release environment, it should be considered that the actual shape of the liquid surface will be different then the release will be different and the strategy change. Please provide a clearer explanation and possible comments about this aspect.

Answer: The oocyst can remain in the solution drop for further analysis and this can be transferred to a multi-well plate as needed (see lines 72 - 73).

Q.2. The explanation about the presence of salt particles in the image must be provided in Fig. 2 that is the first time the reader sees them.

Answer: Fig 2 is modified accordingly. Moreover, related explanation is added to the manuscript (lines 148 -149).

Q.3. In particular in the introduction, when saying “new environment” please specify which environment, for example citing it in brackets.

Answer: This has been added to the introduction section (see line 55).

Q.4. You could avoid citing twice ref 13 in lines 39-41.

Answer: We deleted the first one (see lines 39 - 41).

Q.5. More details should be provided about the vision system calibration. Moreover, what about noise filtering and image distortion correction? Information on the image resolution, field of view, etc. are still missing.

Answer: Noise filtering is mentioned in the top right of Figure 2; greyscale threshold, proper close, fill holes, and particle filter based on area are used to reduce noise in the image and clearly define the boundaries of the objects within the image. These were completed using LabVIEW IMAQ VI’s. The camera has 2592 x 1944 resolution and field of view 179 x 134 micrometers, meaning 14.5 pixels per micrometer (calibrated using the Olympus objective micrometer).

As a high level of accuracy was not required in the current strategy (due to the manual capture), image distortion correction was not implemented. If automated capture was developed then this may be required at a later stage.

Those details have been added to the manuscript in lines 147-150 and 151-153.

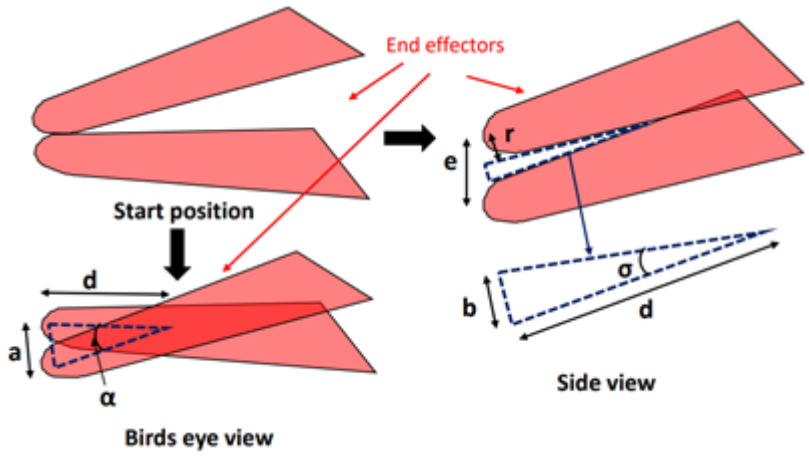
Q.6. It is still not clear to me how accuracy was defined. The mathematical relation computed for accuracy calculation should be provided (lines 175-187).

Answer: Authors believe adding this would not add any value to the manuscript, so we prefer to avoid adding details of this calculation to the manuscript. However, we will provide this info as a supplementary material if any reader is interested to see details of this calculation process (See supplementary material document for details). Summary of the procedure can be found below too.

To test the accuracy of the chosen alignment strategy an experiment was conducted using two end effectors, with the second "passive" end effector placed at a known angle relative to the end effector being aligned. To begin, an additional z axis actuator was used to manually align the passive end effector with the focus plane. The "active" end effector being tested was aligned with the focus plane using the automated alignment method, then actuated along the y axis in increments of 1µm until interference between the two end effectors was observed. The accuracy of the initial manual alignment of the passive end effector was checked in the experiment by ensuring the alignment error did not favour a positive or negative value. The method to check this was to move the active end effector vertically upwards after the experiment and observe if there was increased movement of the passive end effector, in which case it was assumed the alignment error had been negative.

To calculate the alignment error a mathematical model was developed. The following notation is used:

- e – Error of the alignment.
- σ – The taper of the end effectors (the difference in angle between the top and bottom surfaces).
- α – The angle difference between the centroid of the end effectors superimposed on the horizontal plane.
- d – Distance from each end effector tip to the point of first contact between the end effectors.
- a – The y-axis distance between the center of each end effector tip.
- b – The distance between the external edge of the tips of each end effector (assuming the tip has radius r).



Notation used for calculation of the accuracy of the alignment experiment.

Trigonometry was used to find the relationship between the observed overlap of the end effectors (a) and the error in alignment between the end effectors (e). The following assumptions are used to simplify the analysis:

- The d value from each perspective is equal --- this means the point of first contact would lie at the vertical maximum or minimum of each end effector. This is valid when angles σ and α are small.}
- The error (e) is assumed to equal b + 2r. This is valid when the angle between the end effectors and the horizontal plane is small.

Based on these assumptions the relationship between the error (e) and displacement (a) is:

$$e = a\left(\frac{\sin\frac{\sigma}{2}}{\sin\frac{\alpha}{2}}\right) + 2r$$

Q.7. The time calculation was based only on 5 random trials, but it must be performed based on all trials. Comments to the obtained results should be added, similar to those the authors did to reply to this reviewer in Q.28.

Best regards

Answer:

We have looked over all the trials and updated numbers (see table 3 and related explanation in lines 331 - 342). To note is that there were four manual captures that took a much longer time than the average (97, 95, 146, 270 seconds) that affected the mean and standard deviation compared to what was observed for the five random samples.

Answer to Q.28 is added to manuscript now (see lines 331 - 342).