**Quick guideline for using T.U.R.D. software**

The Ultimate Reader of Dung (T.U.R.D.) is a software employed for evaluating the Drosophila intestinal physiology and it was designed by Paola Cognigni and Irene Miguel-Aliaga27. T.U.R.D. can evaluate various physiological parameters, including water balance, fecal output, and acid-base balance by examining the morphology and colour of excreta that Drosophila produce after being fed a dye-supplemented diet.

1. Download the software package: https://sourceforge.net/p/the-ultimate-reader-of-dung/code/ci/master/tree/
2. Install the T.U.R.D. software on the computer.
3. Launch the T.U.R.D. application, a small window appears with a simple toolbar on top (**Supplementary Figure 1**). Create a new experiment by clicking on **File > New** **Experiment** and then give a name to the document.
4. Add a plate (= Petri dish) to analyse. Click on **Plates > Add plate**, choose the Petri dish (already cropped) to work with and select it. A new window appears, which allows to process the plate selected by modifying different parameters as described below (**Supplementary** **Figure 2**).
   1. The block size (pixels) and offset are related to the detection of deposits and light contrast. Change these settings to improve the detection of deposits that are not visible. If the background of the image is dark, reduce the block size from 21 to 7 or increase the offset from 5 to 8. Try several times to find the best settings, which depend on the image's light contrast. If the background of the image is white, set the block size to 45 and offset to 5.
   2. The min size and the max size are two parameters that are expressed in pixels and indicate the minimum and maximum size of deposit detection. If very small spots (not related to fecal deposits) are detected on the scan, increase the minimum size. If too large spots are detected, reduce the max size. If small fecal deposits are not detected, the decrease the min size. The parameters have been tested and seem to be equally effective (in most cases) with min size = 20, and max size = 600.
5. The brush shape is another element to consider. Depending on its value, it can improve the detection of deposits with specific shapes: round, square, oblong. Keep the brush size and circularity threshold as 3 and 0.7, respectively. When the deposit circularity is lower than the circularity threshold defined by the user, it will be color coded by blue numbers.
6. After the software has processed the images, click on **Plates**, then on **Inspect Selected Plates**, then on **Graphics**, and finally on **View annotated images**.
7. Zoom in and look more closely at the detected spots to verify that the fecal deposits detected are the right ones and make sure the software has identified as many fecal deposits as possible (**Supplementary** **Figure 3**). If there are only a few deposits that should not be included in the analysis, deselect the deposits to be excluded (**Supplementary** **Figure 4**). If too many spots are not detected, redo step 3 until the best settings is found.

1. Modify the number of flies and modify the group name by clicking on **Plates > Edit groups > Add**, then in the Group column choose the group name (**Supplementary** **Figure 5**).
2. Export the different replicates data separately by clicking on **Analyze > Descriptive Statistics > Select Group** (**Supplementary Figure 6**). Keep all spreadsheet files in the same folder.