

Comet Assay Instructions

ImageJ Macro to calculate the Comet Assay and display the results.
Based on an NIH Image Comet Assay by Herbert M. Geller 1997
<http://www.nhlbi.nih.gov/labs/education/index.htm>

The macro calculates tail length, tail moment, and % of DNA in the tail.

The image should have a dark background and light comets. The comets should be "on scale" for the calculations to be correct. That is, the brightest pixels of interest should be below 255 on an 8-bit scale.

It is recommended that the user do a camera noise subtraction, flat field correction, and a background subtraction on the images before running the macro.

The user is asked to draw an oval first around the head and then the tail. The shape of the ovals should be adjusted to closely fit the head and the tail. Other shapes than ovals can be used.

Results are placed on the results table. Each comet has two lines of results the first (odd numbered) line has the head values the second (even numbered) line has the tail values. The values are as follows: X and Y are coordinates of the Centroid, XM and YM are coordinates of the Center of Mass, IntDen and RawIntDen are the same and are the integrated density, TailLen is the tail length, TailMoment is the tail moment and %TailDNA is percent of total DNA that is in the tail.

The macro labels the tail of each comet with an accession number. This helps identify the processed comets. This feature can be turned off by opening the macro (Comet_Assay.txt), in the Plugins or Macros folder, and inserting double slashes (//) at the start of the lines: `label =toString (I + 1);` and `drawstring(label, tailx, taily);` then saving the file.

There seems to be some imprecision in the definitions of "center" and "center of gravity" in the literature. Here I define "center" as the "Centroid" - average of the X Y coordinates of all pixels in a selection. This is sometimes called the "center of gravity" (Russ p489*). The "Center of Mass" is the brightness-weighted average of X Y coordinates of a selection. I use Center of Mass of the tail and Centroid of the head in calculating the tail length. The tail length as used here is the distance from the Centroid of the head to the Center of Mass of the tail. It is calculated as the Pythagorean distance between two points (Russ p520*). The tail length variable is called CometLen in the code.

Tail Moment is the length of the tail times the integrated density of the tail. Percent of DNA in the tail is the integrated density of the tail divided by the integrated density of the tail plus the integrated density of the head times 100.

*The Image Processing Handbook, 2nd ed, John Russ 1995 ISBN 0-8493-2516-1.

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