Time-lapse projection of real time video reveals substantial detial of *C. elegans* locomotion on agarose and swimming in liquid culture

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Introduction

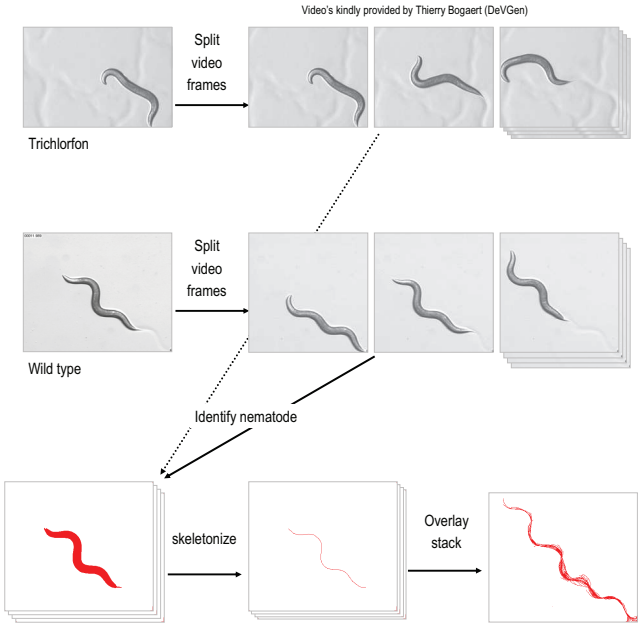
Wild type, mutant and drug-induced locomotion can be scored on agarose in great detail, because of the of the subtle effects that can be discriminated in a 2 dimensional plane at relatively low speed.
In liquid culture in 96- or 384-well plates swimming occurs in 3 dimensions, is more vigorous and therefore less amenable to subtle microscopic scoring.
We aimed at developing software that can reduce the hands-on time needed to evaluate locomotion in genetic or compound screening, using time-lapse video and automated image analysis.

Conclusions

TIME-LAPSE PROJECTION OF LOCOMOTION ON AGAROSE Deconvolution of microscopic video sequences of wild type *C. elegans* and trichlorfon treated N2 into 'hairy rope' images reflect the key features of the video sequence. These images displayed clearly distinct features reflecting wild type and acetyl cholinesterase-inhibited locomotion phenotype similar to the *ace-1, ace-2* double mutant.
SWIMMING IN LIQUID CULTURE Automatic imaging with an interval of 200 milliseconds allows to semi-quantify swimming of nematodes in 96-well plates. This imaging script proved to be very robust in highly variable conditions, and was successfully applied in LD_{50} testing with the nematode on a range of toxic compounds.
We propose 'hairy rope' and motion-difference images as a tool to rapidly evaluate wild type, mutant or drug-induced changes in behavior.

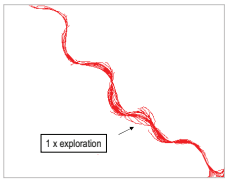
Locomotion on agarose

Assay setup



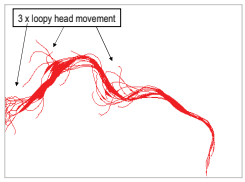
METHODS: Microscopic time-lapse video sequences of wild type untreated and treated C.elegans on agar, were deconvoluted into stacks of images. In each image the nematode was identified by specific image segmentation. The derived binary images were then skeletonized to a line of pixels reflecting the central body axis and projected on each other with different time lapse intervals to create a single 'hairy rope' image.

Results



wild type

sinusoidal track

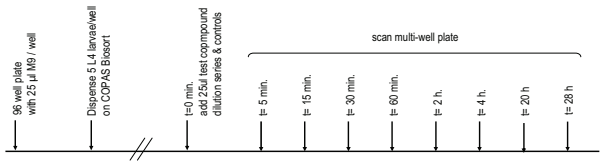


Trichlorfon

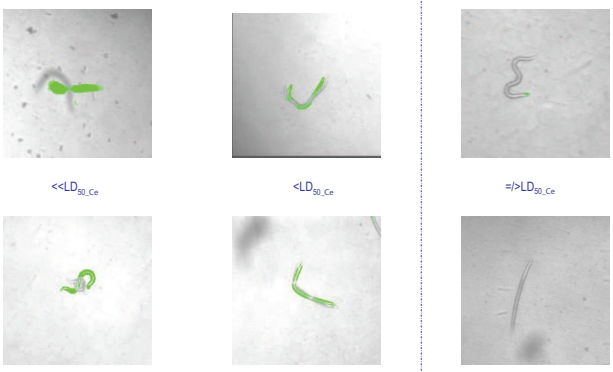
posterior paralyzed (drags body by head)

Swimming in liquid culture

Assay setup



Assay development

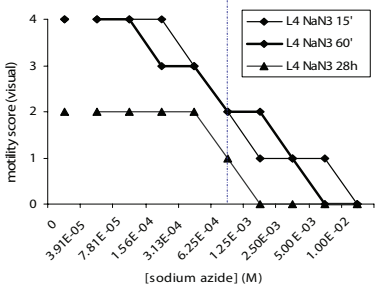


METHODS: L4 larvae were dispensed with the COPAS Biosort into 96-well plates and treated with a dose range (3mM to 0.5 µM) of toxic compounds (NaN_3). The wells were automatically imaged using the MIAS-2 microscopic reader. Two consecutive images with an interval of 200 milliseconds were taken at each scan and locomotion was visualized by subtracting two images and projecting the segmented difference on top of one image in pseudo color.

Results

TO COMPARE*:

- oral LD_{50} for NaN_3 in rat and mouse:
  - 27 mg/kg
- oral LD_{LO} for NaN_3 in man:
  - 29 and 129 mg/kg



* REFERENCE: http://www.bee.cornell.edu/bmb_lab/safety/naazide.html