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APPLICATION

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ToxTrac: A fast and robust software for tracking organisms

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Abstract

1. Behavioural analysis based on video recording is becoming increasingly popular within research fields such as; ecology, medicine, ecotoxicology and toxicology. However, the programs available to analyse the data, which are free of cost, user-friendly, versatile, robust, fast and provide reliable statistics for different organisms (invertebrates, vertebrates and mammals) are significantly limited.
2. We present an automated open-source executable software (*ToxTrac*) for image-based tracking that can simultaneously handle several organisms monitored in a laboratory environment. We compare the performance of *ToxTrac* with current accessible programs on the web.
3. The main advantages of *ToxTrac* are as follows: (i) no specific knowledge of the geometry of the tracked bodies is needed; (ii) processing speed, *ToxTrac* can operate at a rate >25 frames per second in HD videos using modern computers; (iii) simultaneous tracking of multiple organisms in multiple arenas; (iv) integrated distortion correction and camera calibration; (v) robust against false positives; (vi) preservation of individual identification; (vii) useful statistics and heat maps in real scale are exported in image, text and excel formats.
4. *ToxTrac* can be used for high speed tracking of insects, fish, rodents or other species, and provides useful locomotor information in animal behavior experiments. Download *ToxTrac* here: https://toxtrac.sourceforge.io (Current version v2.61).

KEYWORDS

animal behavior, cockroach, ecology, ecotoxicology, guppy, Kalman filter, salmon, tadpole, tracking software, zebrafish

1 | INTRODUCTION

Behavioural assays are frequently used to investigate stress related to social or environmental factors in a variety of research fields within ecology and animal behaviour. Behavioural traits such as activity, sociality, aggression, exploration and boldness are used within animal personality research and studies focusing on evolution driven by environmental changes. (Cote, Clobert, Brodin, Fogarty, & Sih, 2010). Moreover, behavioral assays are becoming increasingly used within ecotoxicology (Brodin et al., 2014) and for medical perspective (Antunes & Biala, 2012). For example, it has been argued that behavioural assays are the most sensitive tool when detecting effects of hazardous substances. Indeed, multiple research fields have a great need to accurately measure organism behavior and many of the unexpected biological effects from xenobiotics in the environment could potentially have been detected before their introduction on the market (and the environment) if behavioral assays had been used in routine chemical risk assessment procedures. Laboratory assays, using visual tracking involving critical behavioural patterns are evolving; however, current methodologies generate huge amounts of data and are often laborious and time-consuming. Available software to analyse this kind of data are costly, slow, often requires programming skills, or are developed as plug-in functions to proprietary programs.

Important features of a tracking software for monitoring animal behaviour are algorithms that accurately can detect the position of the organisms and provide a reliable analysis for long time series. Also, it should allow handling of new experimental setups. In addition, the software should be robust over time and computationally efficient, preferably enabling data processing of several experiments simultaneously. A rather efficient tracking algorithm is the Kalman filter, which also is the most common algorithm used for tracking (Kalman, 1960; Rodriguez, Bermúdez, Rabuñal, & Puertas, 2015). This recursive filter uses the animals previous position to estimate the next, thus reducing the search range and the risk of identification foreign objects as the tracked animals. The main advantages of the Kalman filter are that it allows tracking of multiple objects and it does not require any knowledge of the animal shape. However, the Kalman filter always follows the closest known object to the predicted position, which means that it is very sensitive to false-positives and false-negatives. Additionally, the Kalman filter is unreliable in the presence of occlusions since it does not preserve the identity of multiple animals after occlusions.

Different approaches have been developed to address the problem of tracking multiple animals. Using several cameras can improve tracking, using data from different perspectives to solve occlusions (Maaswinkel, Zhu, & Weng, 2013). However, this adds complexity to the experimental setup and increases the amount of data. Some techniques tag individuals with visual markers to provide a distinct ID to each animal (Crall, Gravish, Mountcastle, & Combes, 2015); other techniques use specific features of the animals such as the symmetry axis (Fontaine et al., 2008) or the shape of the head (Qian, Cheng, & Chen, 2014; Qian, Wang, Cheng, & Chen, 2016). Also model based approaches that use the geometry of the body are suggested (Wang, Cheng, Qian, Liu, & Chen, 2016). The work by Pérez-Escudero, Vicente-Page, Hinz, Arganda, and de Polavieja (2014) is a notable attempt to keep the identities correct overtime. They extract characteristic fingerprints from each animal that are matched with the trajectories in the video and use a resegmentation stage, optimized for particular shapes that reduces the number of occlusions. This approach is, however, very computationally heavy and as such unsuitable for real-time applications, online processing and for bulk processing of huge datasets; it is not scalable with regards of the numbers of animals, the video length or the video resolution; and finally, it requires a minimum size of the individuals and a huge number of samples for each individual to work (3,000 samples are recommended).

Thus, there is a need for a program that is open-source, easy to use and flexible, robust overtime, can handle multiple species, and analyse data fast. In this work, we present *ToxTrac*, a free program for tracking animals in multiple arenas. *ToxTrac* can also track multiple animals reliably handling occlusions and preserving the identity of the individuals. *ToxTrac* uses only the information of the current frame and previously estimated information and the video streaming operations are performed at a nearly constant rate. This makes *ToxTrac* as far as our knowledge goes, the first program of these characteristics fully compatible with a real-time application.

2 | QUICK GUIDE

To install *ToxTrac*, execute the Windows installer file provided here https://toxtrac.sourceforge.io. *ToxTrac* supports a wide variety of AVI video files with any resolution and frame rate. If the video is cut into several files add sequential numbers to the end of file names and placed them in the same folder.

Multiple video sequences can be analysed and multiple animals can be tracked in the same arena, however, we do not recommend, using more than 10–20 animals in a single experiment.

Video resolution should be high enough so that the animal size is at least 50 pixels, and frame rate should be high enough so the animal area in consecutive frames overlap. Often, 25 fps is enough for most experiments.

ToxTrac will detect and track animals inside closed arenas. The tracking areas are uniform bright regions where the animals can be detected. If the arenas have dark corners or edges, exclude these from the tracking area in the program. Ideally, the background colour in the tracking areas should be homogeneous and significantly brighter than the animal. Aim for highest possible contrast.

The software can remove background objects, for example, objects much smaller than the animals can be easily filtered out by changing a parameter related to the size of the animals whereas static objects, of any size, can be removed, using the background subtraction algorithm. Recall that it is important to use a background as free of reflections or shadows as possible and to exclude the dark edges of the experimental setup from the background.

The software has a sophisticated calibration routine that only needs to be done once prior to a set of experiments. However, for this to work perfectly, the experimental setup should be isolated from external interference and light variations. It is important that the experimental setup keeps the same conditions and that it is not moved during an experiment or between experiments.

Finally, make sure you reduce strong shadows or reflections from the arena. This can be achieved by positioning the camera accurately, choice of arena materials, and using a diffuser or backlight illumination strategy. Additional details of how to build a setup and operate the program can be found in the User Guide located here: https://toxtrac.sourceforge.io

3 | PERFORMANCE OF THE PROGRAM

To evaluate the performance of *ToxTrac*, we performed a detailed comparison with other available free tracking tools (see Supporting Information) and measured when possible, the results obtained using *ToxTrac* with that of *IdTracker*, the most reliable open-source tracking software currently available, according to our opinion and tests. We first evaluated the detection rate of *ToxTrac* and *IdTracker*, using eight video datasets from seven organisms (see Figure 1 and Supporting Information). *ToxTrac* and *IdTracker* showed an average detection rate of 99.2% and 95.9%, respectively, in the non-occluded experiments. As expected, when the animals are occluded, that is, when multiple individuals are in the same spot and they are not visually separable,

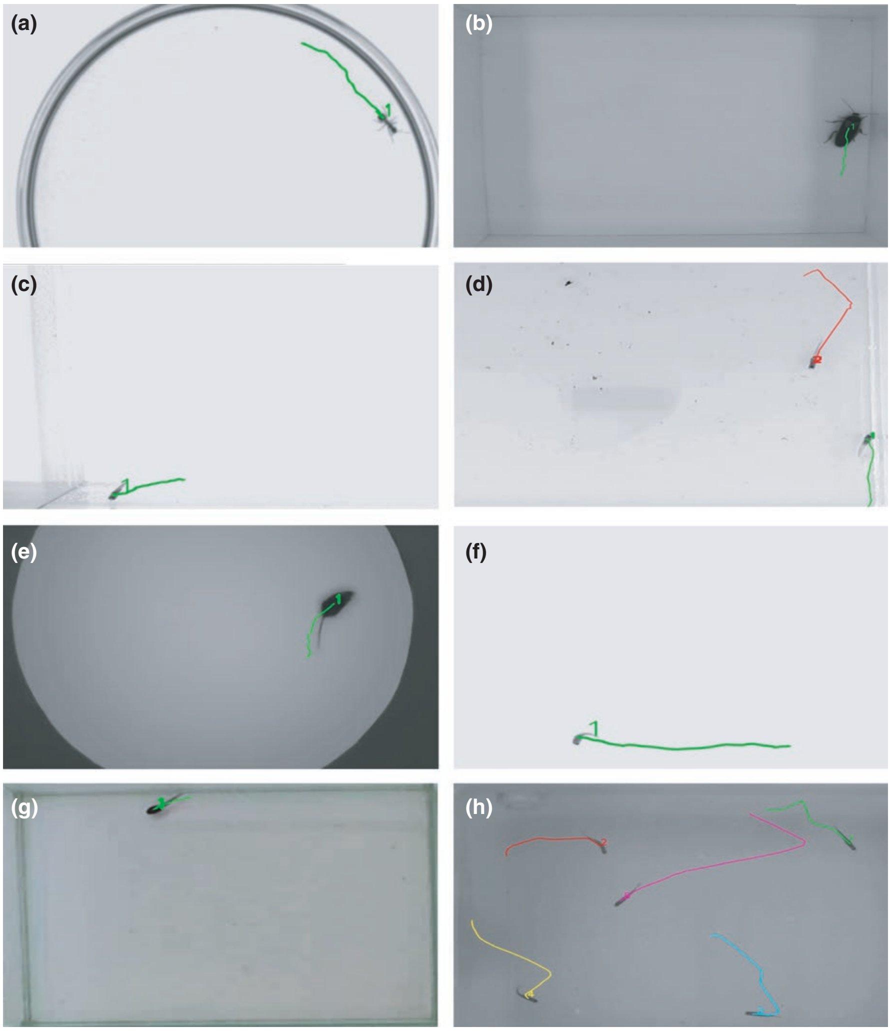

FIGURE 1 *ToxTrac* tracking examples using eight different organisms illuminated using either backlight or direct light. (a) ant, (b) cockroach, (c) guppy 1, (d) guppy 2, (e) mouse (Samson et al., 2015), (f) salmon, (g) tadpole, (h) zebrafish

the detection rate drops accordingly. In this case *IdTracker* obtains better results.

The tracking times were computed for *ToxTrac* and *IdTracker* in the same computer, both algorithms used a 500 reference frames for storing feature information and other parameters were set as similar as possible. We measured the processing times of *ToxTrac* with and without using the fragment identification post-processing step. The tracking times with *ToxTrac* are in all cases significantly lower (see Table 1) details of the validation methodology can be found in Supporting Information.

It should be also noted that with high resolution videos such as the cockroach experiment, *IdTracker* is very slow and usually require the activation of a resolution reduction step. The complete results of this experiment are presented in Table 1.

We also evaluated the fragment identification algorithm. We used the zebrafish dataset (Pérez-Escudero et al., 2014), with five zebrafish showing several crossings with each other. Details of the validation methodology can be found in Supporting Information. Figure 2 shows some examples of how the identification algorithm solves occlusions, and Table 2 shows the complete results of this experiment.

ToxTrac was able to preserve the identity of the tracked animals in 99.6% of the cases when tracks were longer than 50 frames. For shorter tracks, the identification algorithm is less reliable, but still shows a very good accuracy, 92.2%. Though a comparison under the same conditions is not possible with *IdTracker*, due to the differences in the workflow of both algorithms, the reported results of *IdTracker* and other state-of-the-art-techniques using fragment linking algorithms (Pérez-Escudero et al., 2014; Qian et al., 2014) are similar. However, note that *ToxTrac* operates under real-time restrictions (only current frame is used) and has the advantage of being significantly faster, additionally *Idtracker* uses a resegmenation algorithm optimized for a particular animal shape.

Explanation and a flow diagram of the tracking algorithms are found in Supporting Information.

4 | DISCUSSION

Animal behaviour is central in ecological and evolutionary research and is receiving increased attention in many other fields such as ecotoxicology, medicine, neurology and toxicology. Indeed, *ToxTrac* provides several important criteria for becoming a useful tool for

TABLE 1 Tracking performance of *IdTracker* and *ToxTrac* for eight datasets from seven different organisms

	Detection rate		Tracking time		
Experiment	*IdTracker (%)*	*ToxTrac (%)*	*IdTracker*	*ToxTrac (with id. preservation)*	*ToxTrac (without id. preservation)*
Ant	98.25	99.99	00:17:20	00:04:12	00:04:06
Cockroach	99.99	99.99	01:00:30	00:12:20	00:10:54
Guppy 1	96.94	97.65	00:53:38	00:10:42	00:09:23
Guppy 2	99.11	94.52	00:02:44	00:00:06	00:00:04
Mice	99.99	99.99	00:02:13	00:00:15	00:00:14
Salmon	90.55	99.52	01:14:10	00:18:33	00:16:43
Tadpole	99.99	99.98	00:03:16	00:00:18	00:00:16
Zebrafish	93.23	87.36	00:42:32	00:09:53	00:02:31

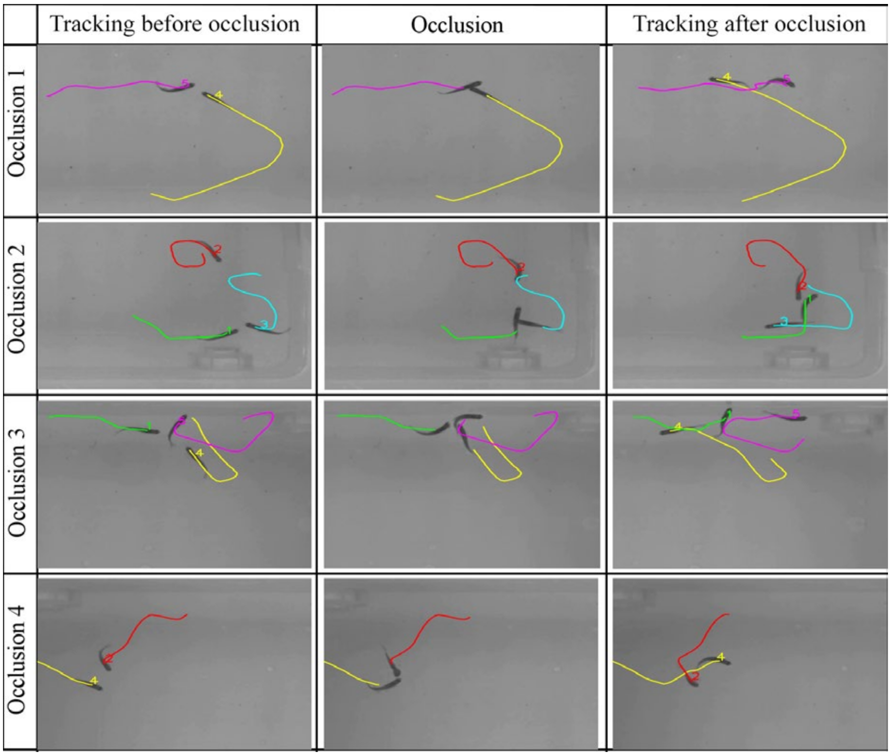

FIGURE 2 Example of occlusions handled by *ToxTrac* from the zebrafish dataset (Pérez-Escudero et al., 2014)

TABLE 2 Identity preservation performance of *ToxTrac*

	Total	Correct id. rate (%)	Incorrect id. rate (%)	Not id. rate (%)
Long Tracks	282	99.65	0.35	0.00
Short Tracks	115	92.17	5.22	2.61
Detections	65,518	99.49	0.37	0.14

these fields being increasingly dependent on animal tracking. *ToxTrac* is a free online tracking software compatible with a real-time application that demands no specific knowledge of the geometry of the tracked objects. It can extract behavioural data from multiple organisms in multiple arenas, integrates distortion correction and real scale calibration measurements, functions robustly against false positives and preserves the identity of individuals if crossing or occlusion occurs.

Some brief examples of endpoints of ecological relevance that *ToxTrac* can measure are outlined below. *ToxTrac* is able to measure behavioural parameters necessary for calculating metabolic rates such as locomotor activity (average speed, acceleration and distance traveled per time unit). The program can also be used to measure the time an organism spends near aquaria or terrarium walls which is a common measure of anxiety within ecotoxicology (Maximino, Marques de Brito, Dias, Gouveia, & Morato, 2010). In *ToxTrac*, the user can adjust the distance of interest to any set object. The flexibility in regions of interest makes the program directly applicable in tests such as scototaxis (dark/light preference), commonly used as a measure of boldness or anxiety but also validated for assessing the antianxiety effects of pharmacological agents (Maximino et al., 2010). The visual plots allows assessment of trajectories that can be used as a measure of how efficient individuals are in exploring novel areas and such data, combined with the generated information of boldness and activity, are necessary when trying to understand how behavioral traits are linked to individual performance or vulnerability to change (e.g. pollution) (Conrad, Weinersmith, Brodin, Saltz, & Sih, 2011). Other useful data provided by the program are mobility rates, and the spatial distribution of the animals in the arena.

The output of all the measures is provided in a widespread format (excel spread-sheet) making it easy for the user to export data into other format necessary for further analysis. The near universal application of the software is also evident from our results showing that the program allows tracking of organisms of very different morphologies and movement patterns (i.e. ants, cockroaches, Atlantic salmon, tadpoles of the common frog, zebrafish and the Trinidadian guppy). In our assays, *ToxTrac* was capable detecting the animals in 99.9% of the frames, which is considered highly acceptable for the common application of behavioral assays. Although *ToxTrac* originally was developed to assay one animal per arena, it is still capable of handling occlusions and multiple animals preserving the identity of the animals using a fragment linking algorithm. Our analysis shows that *ToxTrac* is at least as accurate as other programs used for tracking multiple individuals simultaneously but it process the data significantly faster.

From a practical perspective, *ToxTrac* handles image distortion by using a simple calibration process, which facilitates tracking in environments where, for example, novel objects are co-occurring in images. It also provides bulk processing capabilities, so it is possible to analyse experiments with hundreds of videos in a single step, which greatly facilitate handling large datasets that typically accompany behavioral assays within ecology.

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AUTHORS' CONTRIBUTIONS

All authors designed the study and planned the experiments. A.R. analysed the data and wrote the main manuscript and developed the program. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

The data used to evaluate the program are freely available in the Sourceforge repository (https://toxtrac.sourceforge.io)

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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