idTracker: tracking individuals in a group by automatic identification of unmarked animals

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Animals in groups touch each other, move in paths that cross, and interact in complex ways. Current video tracking methods sometimes switch identities of unmarked individuals during these interactions. These errors propagate and result in random assignments after a few minutes unless manually corrected. We present idTracker, a multitracking algorithm that extracts a characteristic fingerprint from each animal in a video recording of a group. It then uses these fingerprints to identify every individual throughout the video. Tracking by identification prevents propagation of errors, and the correct identities can be maintained indefinitely. idTracker distinguishes animals even when humans cannot, such as for size-matched siblings, and reidentifies animals after they temporarily disappear from view or across different videos. It is robust, easy to use and general. We tested it on fish (*Danio rerio* and *Oryzias latipes*), flies (*Drosophila melanogaster*), ants (*Messor structor*) and mice (*Mus musculus*).

Owing to its high temporal and spatial resolution, video tracking is the main method used in the laboratory to track animals in a group. Tracking systems can follow marked animals in groups for long times[1,2], but marking is sometimes invasive and can modify behavior[3,4]. When animals are not marked, extracting the track of each animal has proven a difficult problem. The source of the difficulty is that when two or more individuals cross or touch, it can be very difficult to find the correct identities after the point of overlap (**Fig. 1a**). Current animal multitracking systems calculate the most likely assignment of identities by taking into account the movement of the animals before and after an overlap[5]. Some of these systems incorporate image processing techniques to separate the images of the individuals when the overlaps are small[6–8] or use species-specific shape models that can help to resolve more complex crossings[8–12]. Some systems use several cameras for three-dimensional (3D) tracking, with the advantage of a smaller proportion of crossings in which the animals overlap simultaneously for all cameras[12–14].

Current methods sometimes assign incorrect identities after a crossing. Even with very low error rates, all animals get random labels after some time because each identity swap in a crossing is an error that propagates to the rest of the video. We illustrated this for a video of eight zebrafish and a simulated tracking system that correctly solves 99% of crossings (**Fig. 1b**). After just 2 min, trajectories had only 11% correct identities. These methods cannot provide labeled trajectories automatically; human operators must review each crossing—a large manual effort[8,11,15].

We have developed idTracker, a software that tracks each animal in a group and maintains the correct identities (**Supplementary Video 1** and **Supplementary Software**; latest software update available at http://www.idtracker.es/). Its methodology is distinct from previous approaches in that idTracker extracts from the video a signature or fingerprint for each individual (**Fig. 1c**). These fingerprints are used to identify individuals in each frame, keeping the correct identities even after crossings or occlusions (**Fig. 1d**). Trajectories are then obtained by joining the centers of the labeled individuals, and an additional algorithm estimates the position of individuals in the regions in which animals overlap. In this way we obtain trajectories with, on average, 99.7% correct identities (**Fig. 1e**; validation against human). The algorithm works for nearly identical individuals, including size-matched unmarked siblings and animals from inbred populations. It works well despite the variability in animal postures and even in cases when the human visual system cannot perform the identification task. The method is 100% automatic and does not suffer from propagation of errors, giving reliably correct identities even for long videos and any complexity of crossings. It is easy to use, needing no modifications for different species (only three parameters must be input by the user; see **Supplementary Note 1**).

RESULTS

Identification in idTracker

The core of idTracker consists in automatically finding a fingerprint for each animal that enables identification throughout the video data (**Supplementary Note 2**). The first step is to extract from the video a set of reference images for each animal. This is done by finding portions of the video in which all individuals are separated. The portion of the video obeying this condition is usually short, as it finishes when any two individuals cross, but the remaining separated individuals can

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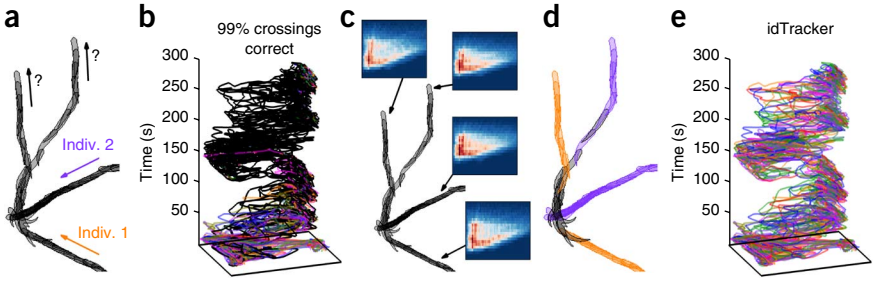

Figure 1 | idTracker maintains correct identities without propagation of errors. (**a**) Silhouettes of two zebrafish whose trajectories cross. If the tracking system switches the identities during the crossing, the error propagates to the rest of the video. A few mistakes then lead to a random assignment. Indiv., individual. (**b**) Trajectories of eight zebrafish (*x* and *y* axes are the sides of the setup; *z* is time) obtained by simulating an algorithm that solves 99% of the crossings correctly. We simulate the error rate by starting from the correct trajectories (validated manually) and switching two identities with a probability of 1% in every crossing. Colors represent correct identities and black represents wrong identity, as compared to that assigned by a human observer. (**c**) Illustration of our method: instead of resolving the crossing, we extract a characteristic fingerprint (insets) that characterizes each individual. (**d**) Same as **a**, with colors representing the identities assigned using the fingerprints. Black indicates that no identification could be performed: for example, when fish overlap. An identification error in one frame (for example, the black frame in the middle of the top purple trajectory could not be identified reliably) does not affect neighboring frames, so errors do not propagate. (**e**) Same as **b**, but showing the results of idTracker. Short errors may occur (they are too short to be seen at this resolution), but they do not propagate.

continue to be used until each has crossed with another. We thus obtain a larger number of reference images for generating the fingerprint of each individual (**Fig. 2a**). Usually this portion of video is still too short to gather enough reference images, especially for species for which crossings are frequent. To increase the number of reference images, the software finds more of these portions of video. It then matches the different portions by aggregating the images that can be assigned with very high probability to the same individual. (**Supplementary Note 2**). At the end of this step, each animal is characterized by a reference set of images with different postures (**Fig. 2b**).

Once the reference images are collected, we identify the remaining images in the video, which we call 'problem images', by comparing them to the references. To perform this comparison, we first transform all images to obtain a clear fingerprint for each animal. For each image of an animal (**Fig. 2c**), after segmentation we obtain the intensities of every pair of pixels (i_1 and i_2) and the distance between them (d) (**Fig. 2d**). This transforms the image into a set of points in the 3D space (d, i_1, i_2), known as the color correlogram of the image[16]. To increase computational efficiency while keeping enough structure for identification, we chose to instead transform the image to the 2D space (d, $i_1 + i_2$). We then obtain an 'intensity map', which is the histogram in this 2D space (**Fig. 2e**), and, similarly, a 'contrast map' for the space (d, $|i_1 - i_2|$) (**Fig. 2f**). These maps give a characteristic fingerprint for each animal (note that it is much easier to distinguish by eye the maps of different individuals in **Fig. 2g** than the original images in **Fig. 2b**). The maps are invariant under translation and rotation, as only intensities and Euclidean distances are used, and robust with respect to changes in posture, as each animal is defined by a reference set with different postures.

The assignment of every problem image to an individual is now done by comparing its intensity map to those of each reference set (**Fig. 2g**). To compare two maps, we subtract them element by element and compute the mean of the absolute values of these differences. Using this metric, we find the reference intensity map most similar to the problem intensity map, and we assign the problem image to the corresponding individual. We do the same for the contrast maps, and if the intensity and contrast maps give contradictory assignments, the assignment is declared

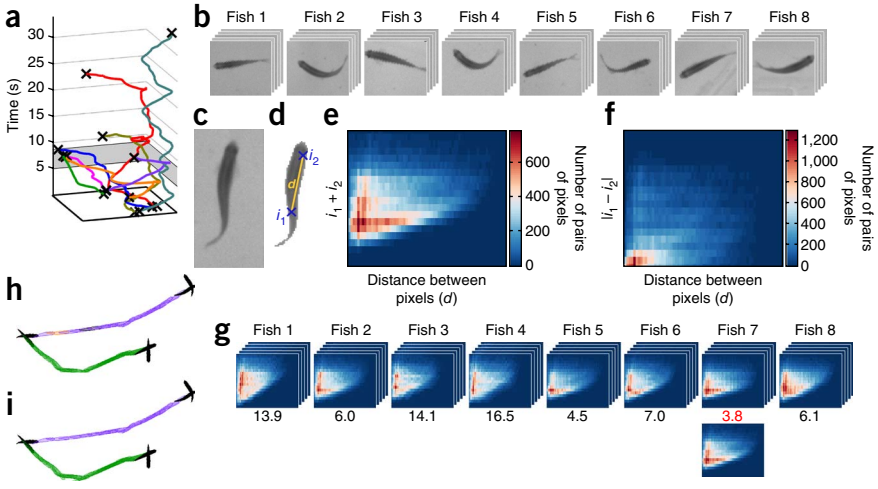

Figure 2 | Identification method. (**a**) Fragments of trajectories for eight zebrafish between consecutive crossings, used to obtain reference images for each animal. *x* and *y* axes are the sides of the setup; *z* is time. Black crosses mark the points at which animals cross. The gray portion corresponds to the period in which all animals are separated. (**b**) Set of reference images that characterize each individual. (**c**) Example of image of one zebrafish. (**d**) Segmented image. We highlight two pixels with intensities i_1 and i_2 separated by a distance d. (**e**) Intensity map of the image in **d**, which is a 2D histogram showing how many pairs of pixels are at a given distance and have a given sum of intensities. (**f**) Contrast map of the image in **d**. It is the same as the intensity map in **e** but using the absolute value of the difference of the intensities instead of the sum. (**g**) Illustration of the identification of one problem image: the numbers are the minimum distance between the intensity map of the problem image (bottom) and the intensity maps of the reference images for each individual (top). The problem image is assigned to the individual with the closest map (fish 7 in this case). (**h**) Silhouettes of two zebrafish as they travel between two consecutive crossings. Colors represent the identification of each image. (**i**) Same as **h**, but colors now represent the overall identification of the whole fragments of trajectories between the two crossings.

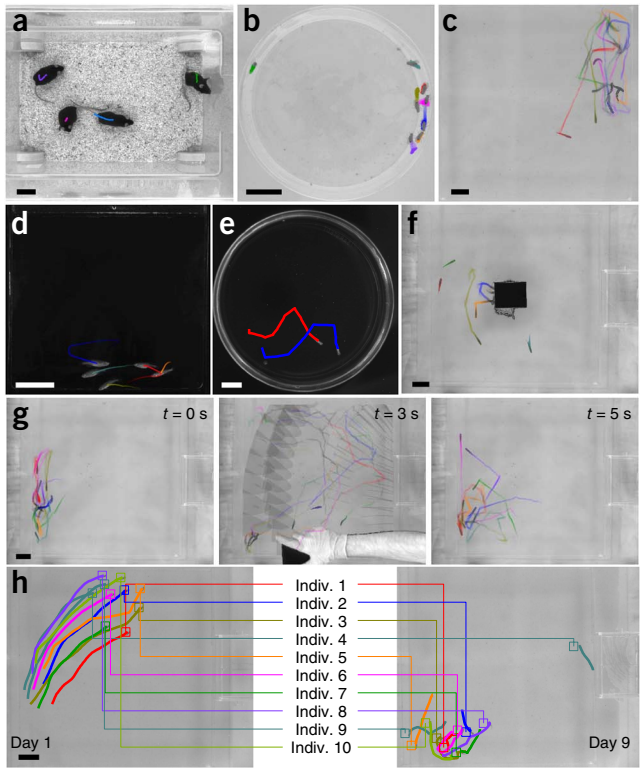

Figure 3 | Examples of applications of idTracker. (**a**–**e**) Frame of a video, with the trajectories provided by idTracker, of mice (**a**), fruit flies (**b**), wild-type zebrafish recorded from the top (**c**) and side (**d**), and *nacre* mutant zebrafish (**e**). (**f**) Same as **a**, for a video of wild-type zebrafish in which a black roof occludes a portion of the setup. (**g**) Three frames of a video of zebrafish in which a hand waves in front of the camera, disrupting the tracking for some time (center). All individuals keep their correct identities before (left) and after (right) the disturbance. (**h**) Frames of two different videos of the same ten medaka fish recorded on different days. The thin colored lines connect the same individual (indiv.) in both videos. Scale bars, 5 cm (**a**,**c**,**d**,**f**–**h**) and 1 cm (**b**,**e**). See also **Supplementary Video 1**.

ambiguous. In order to increase the certainty of identifications, we aggregate the information of all images that belong to the same individual while it moves without crossing with any other individual. This aggregation is an important part of the tracking system: even when some images are incorrectly identified or cannot be assigned (**Fig. 2h**), the final assignment of fragments (**Fig. 2i**) typically has a high probability of being correct. Trajectories joining the centers of the identified animals would leave gaps in the regions with animal crossings. We fill these gaps using an algorithm that estimates the position of each animal starting from the identified images before and after the crossing and working toward its center (**Supplementary Note 2**). The output of the system includes the trajectories and an estimation of the probability that each frame is correctly assigned. This output indicates which portions correspond to estimated positions during animal overlaps so that users can decide whether to include them, depending on the characteristics of the analysis.

Validation against human performance

To test the performance of idTracker under laboratory conditions, we applied it to 23 videos of five different species (**Supplementary Table 1**): mice (*M. musculus*) (**Fig. 3a**); fruit flies (*D. melanogaster*) (**Fig. 3b**); zebrafish (*D. rerio*) from above (**Fig. 3c**) and from the side (**Fig. 3d**); a zebrafish strain that is transparent owing to a *nacre* mutation[17] (**Fig. 3e**), for which eyes and internal organs are enough for identification; a WIK line of zebrafish inbred for five cycles; ants (*M. structor*); and medaka fish (*O. latipes*). We manually validated identifications in portions of video with no animal overlaps and found a mean performance of 99.8% correct trajectories and no error propagation. Mistakes occurred only when the distance between two consecutive crossings was very short (typically shorter than one body length; **Supplementary Note 3**). Different species displayed different proportions of video with image overlaps (from 1% in medaka to 20% in some of our videos of mice). We also performed manual validation during animal overlaps, finding that the estimated position is inside the body of the animal in 96.5% of the trajectories on average. The mean performance for the complete videos, including all portions with and without overlaps, was 99.7% trajectories correct (99.7%, >99.9%, 99.3%, >99.9% and 99.8% for zebrafish, flies, mice, medaka and ants, respectively; each percentage is averaged across all videos of each species).

We further tested that the system maintains its performance for common laboratory conditions and manipulations. In cases in which animals disappeared from view because they were occluded by objects or because they left the camera's field of view, idTracker kept the correct identities before and after they disappeared—for the software these events are identical to animal overlaps (**Fig. 3f**). It was robust to manipulations in the middle of a video, both to additional occlusions (**Fig. 3g**) and to small changes of the animals due to handling (**Supplementary Note 3**). We also found that the system was robust to modification of behavior in the middle of an experiment (**Supplementary Fig. 1**). Additionally, we validated that references obtained in one experiment could be used for several days, allowing identification of individuals across different videos (**Fig. 3h** and **Supplementary Fig. 2**). For these capabilities, our system outperformed not only state-of-the-art methods but also human operators, as they are unable to make such identifications.

In contrast to humans, idTracker finds a signature for each animal using a very distributed representation of their images, taking into account relations between pixels that are at any distance from each other. Humans typically focus on more local features, which might explain why idTracker outperforms humans; moreover, individual animals may have some particular local features that are found by idTracker but cannot be distinguished by humans. To search for these possible features, we studied which pixels were helping idTracker most in the identification. We found that most pixels contributed positively to identifications and that the pattern of contribution was different for different images, even if these images belonged to the same individual (**Supplementary Note 4**). This is consistent with the idea that the distinguishing elements are not local but distributed.

Finally, we evaluated how well the program estimates the tracking quality. We artificially deteriorated some videos and found that in all cases the program distinguished good from bad performance. We also checked that the estimated frame-by-frame probability was a good indicator of the actual probability of correct identity (**Supplementary Fig. 3**).

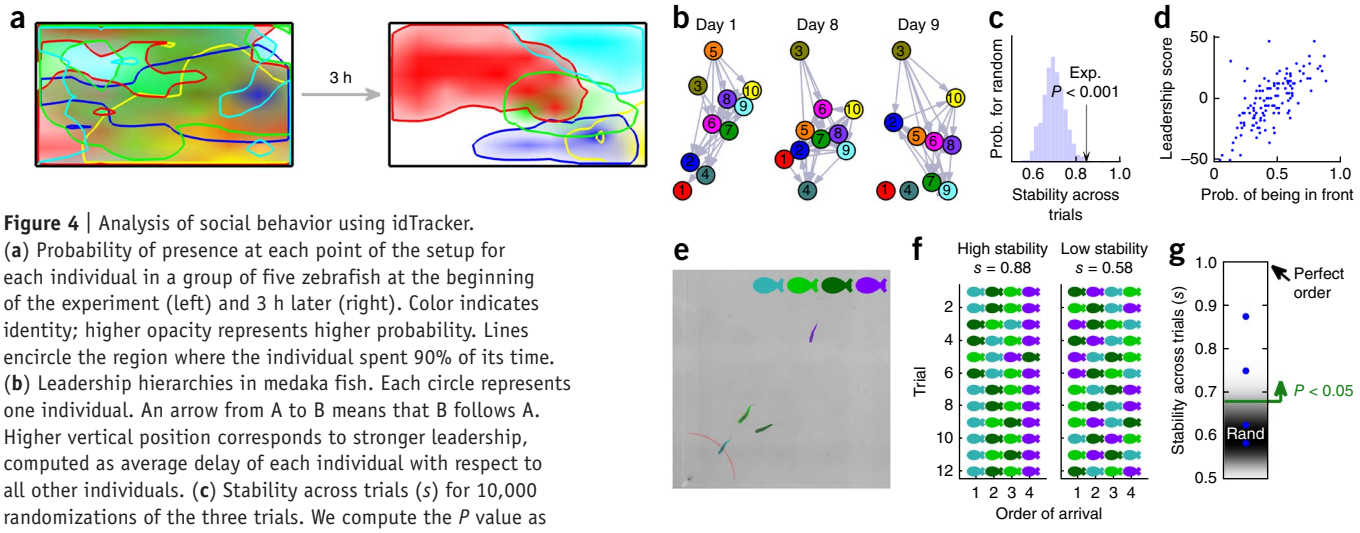

Figure 4 | Analysis of social behavior using idTracker. (**a**) Probability of presence at each point of the setup for each individual in a group of five zebrafish at the beginning of the experiment (left) and 3 h later (right). Color indicates identity; higher opacity represents higher probability. Lines encircle the region where the individual spent 90% of its time. (**b**) Leadership hierarchies in medaka fish. Each circle represents one individual. An arrow from A to B means that B follows A. Higher vertical position corresponds to stronger leadership, computed as average delay of each individual with respect to all other individuals. (**c**) Stability across trials (*s*) for 10,000 randomizations of the three trials. We compute the *P* value as the proportion of randomizations with higher stability than that of the experimental (Exp.) data (arrow). (**d**) Strength of leadership versus probability of being at the front, for all pairs in the group of ten medaka fish. (**e**) Frame illustrating one trial of a food-finding task in zebrafish, with colors representing identities. Silhouettes on the top indicate order of arrival. Upon reaching a threshold (red arc), a fish is considered to have arrived at the food. (**f**) Order of arrival for each trial for two of four total groups, one group with consistent order (left) and another compatible with random order (right). (**g**) Stability across trials for the four groups (blue dots). The black gradient shows the distribution of *s* for random ordering (darker for higher probability). *P* value is computed as for **c**. Script and data to reproduce this figure are in the **Supplementary Data**.

Conditions for the system

Setups for idTracker are easy to build and inexpensive. It runs on personal computers (minimum 8 gigabytes (GB) random access memory (RAM)) with a computation time of 0.5–2 s per frame for the videos tested in **Supplementary Table 1**, with 0.5 s per frame for a video of two *nacre* mutant zebrafish and 2 s per frame for a video with 20 medaka fish. idTracker can analyze videos obtained with a regular camera that records either in uncompressed format or with high-quality compression (many modern consumer video cameras meet this requirement).

Resolution must be high enough to allow identification of the animals. Our validations used at least 150 pixels per animal (**Supplementary Table 1**), corresponding to arenas with sides of 15–25 body lengths, and a camera with resolution of 1,080 × 1,080 pixels. We also studied the limits of the system by resampling some of the videos at lower resolutions. We found for flies, zebrafish and mice that the system had good performance down to 50 pixels (**Supplementary Fig. 4**). But the limit will depend on the conditions of the video, and in general we recommend using at least 150 pixels per animal.

The system requires homogeneous illumination but is robust to small inhomogeneities. Strong inhomogeneities may degrade the tracking at least in one region of the setup (**Supplementary Fig. 5**). Also, animals should have enough contrast against the background to make their segmentation possible (as in **Fig. 3**).

The maximum number of animals we have tracked as a group is 20, but this number depends on species and conditions. Using images of individuals from several videos, we estimate very small deterioration of identifications for up to 35 animals (**Supplementary Fig. 6**). Extrapolation from these results suggests good performance also for larger numbers. Therefore, the limiting factor for tracking a high number of animals is not identification. Instead, the practical limit is currently imposed by the way in which the method extracts references, which can be compromised at high densities if crossings are ubiquitous. Extracting references also requires a minimum length for the video: for the cases tested, at least 5 min and typically 30 min. For tracking a very high number of animals or for very short videos, it is possible to obtain references in separate videos of subgroups of the animals and/or of longer duration.

The program automatically estimates the quality of the output and warns the user when the estimation gives a low quality. Users can use this estimate to confirm the validity of their experimental conditions.

Applications to the study of group behavior

Despite the growing interest in group behavior[15,18–26], its study in the laboratory has met with the difficulty of tracking individuals in groups. To illustrate how idTracker can overcome these difficulties, we used it to study three different problems: spontaneous emergence of territoriality, stability of leadership hierarchies, and differences among individuals of a group when solving a task (raw data and scripts in **Supplementary Data**).

Taking advantage of idTracker's ability to maintain individual identities indefinitely, we studied groups of adult zebrafish for 3 h. The fish shoaled at the beginning, together visiting the entire tank. Afterwards, each animal covered different parts of the tank, and after 3 h, their territories were very different (**Fig. 4a**). Thanks to the ability to track with correct identities for any length of time, it is possible to identify nonstationary aspects of behavior such as this spontaneous switch from shoaling to territorial behavior.

idTracker also allowed us to study whether each animal consistently showed the same type of interactions with other members of the group in different trials. Using a video of ten medaka fish, a species that shows very clear group structure, we studied the network of leadership-followership relations (in each pair a follower is defined as the one with more tendency to copy the velocity vector of the other[26]; Online Methods). We found a hierarchical structure of leadership-followership relations (**Fig. 4b**). idTracker reidentified the individuals in another two videos of the same ten

medaka fish 1 week later (days 8 and 9; see Online Methods for the algorithm to match identities across different videos). The structure was preserved for many of the individuals across the 3 d (**Fig. 4b**). To test whether this result is significant, we defined a stability score (*s*) that is higher the more preserved the hierarchy is across trials, with a value of 1 when each individual has the same rank in all trials (Online Methods). We compared the stability score of the experimental hierarchies with 10,000 randomizations of the experiment, finding that the stability of the actual hierarchies was highly significant ($P < 0.001$; **Fig. 4c** and Online Methods). We also tested whether leaders occupied a particular position within the group: for each pair of fish, we selected one individual at random as a reference individual and computed a leadership score that increases with the strength of the leadership of the reference individual on the other member of the pair (Online Methods). We found a very strong correlation between this score and the probability to find the reference individual in front of the other one, indicating that leaders tended to be at the front of the group (**Fig. 4d**; linear correlation $P < 10^{-10}$).

We performed another set of experiments using groups of four zebrafish to study how a group solves a task, here locating a food patch (**Fig. 4e**). idTracker was used to obtain the tracks of the four animals, reidentifying them in each of the 12 trials. We performed the experiment with four different groups and studied the order of arrival to the food in each trial (**Fig. 4f**). We computed the same stability score (*s*) as for the hierarchies of medaka to measure how consistent the order of arrival was across several trials (Online Methods). We found a diversity of styles in the groups: two groups were highly ordered, and the other two had an arrival order compatible with the random case (**Fig. 4f**,**g**).

DISCUSSION

The development of idTracker has been possible because of four novel elements worth making explicit so as to consider future improvements. First, we have discovered that animals can be individually identified even when humans cannot perform this identification task. Previous tracking methods, probably copying the style in which humans track animals from video, attempted to follow animals in crossings but suffer from error propagation. After finding that animals could be distinguished, we needed three technical developments to obtain an effective tracking from a single video. One is the transformation of the image of each animal into a space in which animals can be easily identified, even for animals that change position and posture. Our intensity and contrast maps are one way to do this, but it should be interesting to explore other local and distributed methods[27–31], including methods that take advantage of characteristic features of some species, such as patterned skins[31]. A second technical development necessary to track animals from a single video is an automatic procedure to extract references for each animal. We developed a method to obtain a set of frames of the same individual in a way that prevents confusion with other individuals. This set of frames for each individual is used as a reference set with no need for extra videos for each individual. This algorithm may be exported to other systems that use extra reference videos[1]. A third technical development is a system to aggregate the frame-by-frame assignations into a final global assignation, its corresponding trajectory and an estimation of the probability of having a globally correct assignment.

Currently the system uses only intensity information (color images are transformed to grayscale before processing). Color might be important for some conditions, both to facilitate segmentation and to improve the quality of identification, but it will probably increase the computational load of the method. If we use the same rationale as for our maps, a color image with three channels will transform into a 7D space (three intensities per pixel of the couple, and the distance between them). In general, an efficient use of the color information will probably require a final space of higher dimensionality than the one we are currently using (either a higher number of 2D maps or higher-dimensionality maps). Another alternative is to automatically adapt the transformation to each video, depending on the type of color information relevant for identification.

Although we have developed idTracker to work for unmarked individuals, it can also be used to distinguish marked individuals. Markings may allow the system to track an even higher number of animals beyond the limit of video resolution and quality imposed by the small differences existing among unmarked animals. In this context, idTracker may contribute with two key advantages with respect to other systems developed for marked animals. First, it can distinguish marks that are smaller and more subtle, allowing for easier marking methods. Second, it automatically extracts the reference images from the video, whereas other systems require a separate video of each animal to learn the structure of marks[1].

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

A.P.-E. designed the project, performed experiments, analyzed data, wrote and tested the software and wrote the paper; R.C.H. performed experiments, tested the software and analyzed data; J.V.-P. performed experiments, tested the software and analyzed data; S.A. performed experiments and tested the software, G.G.d.P. designed the project, advised and directed the project including experiments, software and data analysis and wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

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ONLINE METHODS

Tracking algorithm. idTracker is programmed in Matlab 2010a (The MathWorks), with some routines in C to increase computational speed. The following paragraphs give a brief summary of the main steps of the algorithm; see **Supplementary Note 2** for a complete description of all steps and **Supplementary Software** for the full code (an updated version can be found at http://www.idtracker.es/).

Segmentation. First, each frame is normalized to its mean intensity to control for fluctuations of illumination. To distinguish animals from the background, we select blobs whose pixels have a normalized intensity below (or above) a certain threshold and whose area is larger than a minimum size. An optional background removal routine computes the average image of the whole video and discards pixels that pass the threshold in the average image.

Fragments of trajectories. When an animal is moving without crossing with any other one, we group all its images into a 'fragment' so that we can identify them together, increasing the certainty of the identification. To be sure that we only group images that belong to the same individual, we use a restrictive criterion: two blobs of consecutive frames belong to the same fragment if they overlap with each other and none of them overlaps with any other blob.

Transformation of images. Images are transformed into contrast and intensity maps as described in the main text (**Fig. 2**).

Selection of images that belong to a single individual. To distinguish blobs that belong to one individual from blobs that belong to several overlapping individuals and from noise, we extract a small collection of single-individual blobs from the video. To extract this collection we assume that if a frame contains a number of blobs equal to the number of individuals in the video, each of these blobs belongs to one individual. Then we compare the maps of all blobs with the collection of single-individual blobs as described in the main text (**Fig. 2**). Blobs whose difference with the collection is compatible with the differences within the collection are classified as single-individual blobs. We define single-individual fragments as those with a majority of single-individual blobs.

Collection of reference images. First, we look for periods of time when all individuals are separated (there are simultaneously as many single-individual fragments as animals). Each of these periods provides us with a set of fragments, each of them belonging to one of the individuals. To aggregate several sets of fragments, we compare them pairwise. For each pair of sets of fragments, we use one of them as 'reference set' and identify all the blobs of the other set ('problem set') as described in the main text (**Fig. 2**). With these identifications we compute the probability that each of the fragments of the problem set belongs to the same individual as each of the fragments of the reference set (probability P_2 in **Supplementary Note 2**). To increase the certainty of the relations, we use the fact that they must be consistent: If we have three sets of fragments, the relations of identities between sets one and two and sets one and three determine a unique relation between sets two and three. Using this fact we compute an aggregated probability (P_3 in **Supplementary Note 2**). Because there are typically several dozens of sets of fragments, this aggregated probability gives very certain assignments, even when each individual pairwise comparison may not be very reliable. We aggregate all sets of fragments that can be linked with error probability lower than 10^{-5}, up to a maximum number of reference images (typically 3,000).

Identification. We compare each blob with the reference images, as described in the main text (**Fig. 2**). We then aggregate the identifications of all blobs of the same fragment, computing the probability for each identity for that fragment (P_1 in **Supplementary Note 2**). Taking into account that two blobs at the same frame must belong to different individuals, we aggregate the probabilities of all fragments (computing probabilities P_2 in **Supplementary Note 2**). We then take the fragment whose identity has highest certainty, assign it to its most likely identity and reset its probabilities as 1 for the assigned identity and 0 for the rest. We then update the probabilities of all other fragments and repeat the process until all fragments have been assigned. The aggregation of information in this process, and the assignment from most certain to less certain (as opposed, for example, to a chronological assignment) greatly increases the accuracy of the final identities.

Matching identities across videos. To match the identities of the same individuals across different videos, we use the same procedure as that to match different fragments in the same video when we build the references (see above and **Supplementary Note 2**). After finding the most likely assignment between the videos according to the probability P_3, we compute the probability of the assignment as the probability for the most uncertain individual. We reject the video whenever the probability of mistake is higher than 10^{-10}. We include a validation of this procedure in a case in which we know the true identity of the individuals (**Supplementary Fig. 2**). For all the experiments shown in **Figure 4**, error probabilities were below this threshold, and we checked that the redundant assignments were consistent, as illustrated in **Supplementary Figure 7** for the three videos of ten medaka fish.

Computer. We have tracked the videos on a laptop computer Toshiba Satellite R630 (processor Intel Core i5, 8 GB RAM, Windows 7 64 bits) or desktop computer (processor Intel Core i7-2600, Windows 7 64 bits, 8 GB RAM).

Animal rearing and handling. All procedures met with European guidelines for animal experiments under Directive 86/609/EEC. Experimental procedures were approved by the Bioethics Subcommittee of Consejo Superior de Investigaciones Científicas (CSIC, Spain). For zebrafish (*D. rerio*), we used a stable line obtained in the lab using a pair of siblings obtained from animals bought at a local pet store, a WIK line we have inbred for five cycles to obtain close to isogenic animals and a *nacre* line[17], a gift of G. Sumbre. For medaka (*O. latipes*) we obtained a laboratory line, gifted by P. Bovolenta. Fish were kept in the animal facility with a 14/10 light/dark cycle, in 5-l or 8-l transparent containers connected to a larger fish rack system with circulating water at 26.5 ± 0.5 °C and at 7–7.5 pH. Fish densities were up to 1.25 fish

per liter. Fish were fed live artemia (*Artemia salina*) twice a day, and fish flakes (Sera Vipal) once a day. Water conditions were maintained using appropriate filters and were measured once a week in order to keep low levels of NH_3, NO_2^- and NO_3^-. Fish larvae (*D. rerio* and *O. latipes*) were reared in 150-mm Petri dishes and were fed twice a day with dry food (Sera Micron) and liquid food (JBL Nobil Fluid). Petri dishes were cleaned, and half of the water was changed once a day. At day 15, larvae were moved to the animal facility. Mice (*M. musculus*) of the C57BL/6 and Agouti strains were kept in groups of two to five individuals in standard mouse cages under a 14/10 light cycle, with *ad libitum* access to food and water, and under constant humidity (55% ± 10%) and temperature (22 ± 2 °C). Fruit flies (*D. melanogaster*) of the Canton-S strain were kept in tubes with *ad libitum* access to standard cornmeal food, under a 14/10 light cycle. An unidentified local species of ant was collected for behavioral experiments and returned afterwards. *M. structor* were available from the animal facilities of the Research Center on Animal Cognition (CNRS - UPS Research Institute no. 5169), where they are under a 12/12 light/dark cycle and fed three times a week with regular ant-rearing food[32].

Territoriality in zebrafish. We used adult fish of both sexes. Fish were acclimatized to the water of the behavioral setup 2 d before starting experiments. We recorded a group of five zebrafish in a 47 × 26 × 2.5 cm^3 behavioral tank (width × length × height) at 27–28 °C. As the experiments were 3 h long, we took special care in maintaining the quality of water: the bottom of the behavioral tank consisted of a transparent and permeable mesh, which facilitated a steady exchange of water with an acclimatized large external container. We recorded a 20-min video at the beginning of the experiment and another one 3 h later. We used idTracker to extract the trajectory of each individual and to relate the identities between the two videos. **Figure 4a** corresponds to intervals between minute 10 and minute 20 (left) and between minute 180 and minute 190 of the experiment (right).

Leadership/followership hierarchies in medaka. We used adult fish of both sexes. Fish were acclimatized to the water of the behavioral setup 1 h before starting experiments and returned to the animal facilities every day after recording. We recorded a group of ten medaka fish in a tank of 50 × 50 × 2 cm^3 (width × length × height), the same setup used in validation videos (see below). The fish entered the setup through a door at one side and swam freely for 30 min. Both social cohesion and activity decrease with time, so we analyzed only the first 3 min of the experiment. We measured the leadership/followership relationship between every pair of individuals i and j using delayed correlations in direction of motion[26] as

$$C(\tau) = \langle \vec{v}_i(t_k + \tau) \cdot \vec{v}_j(t_k) \rangle$$

where $\vec{v}_i \cdot \vec{v}_j$ is the scalar product of the unit velocity vectors for individuals i and j, respectively, τ is a time delay and $\langle\ \rangle$ means average over the times t_k in which the two individuals are separated less than 10 cm and move faster than 3 cm/s (this speed corresponds to 2 pixels per frame in our videos and is the minimum for which we can measure the direction accurately). The scalar product measures the alignment of the velocity vectors, giving 1 when they are perfectly aligned, −1 when antialigned and 0 when perpendicular to each other. The rationale for this analysis is that if a leading individual changes direction, the follower will copy this change after some delay. Therefore, we should find a better alignment after some delay. We scanned delays between −2 s and 2 s and found the delay τ_m for which $C(\tau)$ is maximum. If $C(\tau_m)$ was higher than 0.6, we considered that one animal is following the other, and for positive (negative) τ_m, individual i (j) is the follower of the pair. Assuming similar reaction times for all fish in the hierarchy, a large delay τ_m between two fish indicates that there is an intermediate fish between them in the hierarchy. Therefore, delay is a good proxy of position in the hierarchy. Higher vertical position of each individual in the hierarchy thus corresponds to stronger leadership computed as the average delay of each individual with respect to all other individuals for which leadership/followership is significant. We define the 'leadership score' between every pair as the delay τ_m between them (**Fig. 4d**). To compute the probability of being in front, we take into account all pairs of fish that have more than 100 frames in which both fish move faster than 3 cm/s and $\vec{v}_i \cdot \vec{v}_j$ is higher than 0.9. Then we project positions of the two fish along a vector in the average direction of both fish ($\vec{v}_i + \vec{v}_j$) and compute the proportion of frames in which the projected position of fish j is in front of the projected position of fish i.

Food-finding in zebrafish. We used adult fish of both sexes. Fish were acclimatized to the water of the behavioral setup 1 d before starting the experiments. Fish remained in this water for the whole duration of the experiment, a 100-l tank equipped with water acclimatizer that kept temperature at 26.5 ± 0.5 °C. We kept each group of four fish in a plastic box submerged in the larger tank, with holes to allow water circulation. Four groups of four fish take part in this experiment. In each trial, the four fish of a group enter the behavioral tank (50 × 50 × 2 cm^3, width × length × height) through a door in the middle of one side. Two pipette tips were held at the corners of the opposite side. One of the tips was covered by fish food (Sera Vipal flakes glued with agar to the pipette tip), and the other was clean. The tip with food was always at the same side in all trials for the same group (two groups were trained with food on the left, and the other two with food on the right). We allowed fish to swim freely in the setup for 5 min. We performed 2–4 trials per day and a total of 28 trials: 15 initial trials with food, 1 trial without food and mixing two groups previously trained to go to different places (animals were then unmixed using idTracker) and another 12 trials with food. We present the analysis of the last 12 trials (**Fig. 4e–g**). We used idTracker to follow the trajectory of each individual and to relate their identities across trials. We considered that an individual arrives to the food when it reaches the region at 8 cm from the tip with food.

Stability of ordering across trials (s). This score measures how consistent the ordering of a group is across several trials. First, we computed the average rank of each individual across all trials. Then, for each trial and each pair of individuals of the group, we added 1 if the individual with higher rank in the current trial also had higher average rank. We defined s as the resulting number divided by the number of trials and the number of pairs. Thus, s = 1 if all trials have identical ordering. For random ordering, s tends to 0.5 in the limit of a large number of trials.

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To compute the *P* value of a given experiment, we generated 10,000 random repetitions of the experiment. To generate each random repetition, we permuted randomly the order of the individuals in each trial. Then we computed the *P* value as the proportion of random repetitions whose value of *s* is equal or higher than the experimental one.

Setups for validation videos. This section describes the setups in which we have tested the tracking system. The conditions described here must not be understood as necessary (we have successfully tracked videos recorded by other researchers in different conditions; see the main text and **Supplementary Note 1** for a general description of the conditions required).

Unless indicated otherwise, videos have been recorded with a monochrome Basler A622f camera that has a resolution of 1,280 × 1,024 pixels. It is connected to a computer via Firewire (IEEE 1394) and at full resolution has a frame rate around 25 f.p.s. (the frame rate increases when we decrease resolution using only one part of the camera's sensor. We typically selected a region of interest that fits tightly on the arena, so most videos were recorded at higher frame rates, see **Supplementary Table 1**). The videos were directly recorded on the computer's hard disk, their length being limited only by the hard disk capacity (around 20 h of uncompressed video on a 2-TB hard disk). The rest of the setup was as described below for each species.

Setup for zebrafish (*D. rerio*) and medaka fish (*O. latipes*). We used two different setups to record fish, the main difference being that for some videos we placed a transparent cover between the fish and the water surface to prevent the formation of ripples. We found that this cover is not necessary in general but convenient for large groups of the fastest-moving fish (i.e., zebrafish).

The setup with cover was used for all videos of zebrafish and medaka, except the one labeled 'without cover' (**Supplementary Table 1**). It consisted of a 50 × 50 × 2 cm^3 (length × width × height) arena made from transparent Perspex. The height of the arena is enough for the fish to behave normally, with multiple fish easily crossing one on top of each other. This arena was completely closed, including a transparent roof, but was not watertight. It was fully submersed into a larger tank (90 × 120 × 20 cm^3) equipped with a water acclimatizer to maintain healthy conditions for the fish. The arena was sustained by four legs at around 5 cm above the white floor of the larger tank so that the shadows of the fish were diffused, facilitating the segmentation. The camera was situated over the setup at a distance of about 1.2 m, pointing directly downwards and equipped with an objective of 16-mm focal length, Pentax C31634KP - C1614-M (KP). In these conditions, the setup covers around 950 × 950 pixels in the image. In order to have indirect and uniform illumination, we used six halogen floodlights (500 W each) pointing to the ceiling. A brown cardboard surface of 120 × 150 cm^2 at the level of the camera prevented the light directly reflected on the ceiling to reach the setup so that illumination reaching the setup was indirect. Also, this brown surface projected a dim and uniform reflection on the water surface.

For the video of five zebrafish without cover, we used a 62 × 45 × 18 cm^3 (length × width × height) translucent plastic box with no roof, filled with water up to 3 cm. We placed this box into the same larger tank equipped with water acclimatizer as in the previous case, keeping the floor of the plastic box at around 10 cm above the white floor of the larger tank. Illumination and camera disposition were the same as for the previous setup. In this case, the setup covered around 1,150 × 810 pixels in the image.

Setup for juvenile nacre zebrafish (*D. rerio*). We used a Petri dish of diameter 8.5 cm covered by a transparent Perspex lid and fully submerged in water. We placed a black plastic below the dish to increase contrast. Illumination and camera were the same as before, but with the camera closer to the setup.

Setup for zebrafish (*D. rerio*), recording from the side. We used a 25 × 3 × 25 cm^3 (length × width × height) chamber inside a bigger tank made of glass. The camera was at 1 m of the setup, pointing horizontally toward the 25 × 25 cm^2 face of the setup. Illumination was the same as for the previous setups. Black background gives the best contrast in this case, so we placed a black curtain over the rear side of the tank. We also placed a black curtain around the camera's objective in order to obtain a dim and uniform reflection from the glass tank.

Setup for mice (*M. musculus*). The videos with four mice were recorded inside a translucent plastic cage of size 30 × 47 × 35 cm^3 (length × width × height). It has no roof, the walls being high enough to prevent the mice from escaping. Videos with two mice were recorded in a transparent plastic cage of size 18 × 32 × 20 cm^3 covered with a transparent Perspex roof to prevent the mice from escaping. In both cases, the bottom of the cage was covered with sawdust for comfort of the animals. Camera and illumination were the same as for the fish setup, with the camera at around 110 cm and 100 cm from the floor of the setup for the four-mice and two-mice videos, respectively.

Setup for flies (*D. melanogaster*) and locally collected ants. The floor of the arena was made of transparent Perspex. Walls and roof consisted of a Petri dish of diameter 5.5 cm placed upside down. The inside of the Petri dish was coated with Fluon (polytetrafluoroethylene, Sigma-Aldrich product number 665800). Fluon is slippery for most insects, preventing them from climbing the walls and roof. With this configuration we could record walking flies with no need to cut their wings. The camera was placed 10 cm below the setup, pointing upwards. We used a Pentax C31635KP - C1614-5M (KP) (focal length 16 mm), which can focus at such a short distance. The insects were therefore seen from below through the transparent floor against the white background of the Petri dish covered with Fluon. The setup was surrounded by white curtains, and illumination was provided by five halogen floodlights (500 W each) outside the curtains and pointing toward the inside.

Setup for ants (*M. structor*). The ants were inside a circular box of 10-cm diameter with white floor and walls and without roof. We covered the walls with Fluon to prevent the ants from climbing them. It was located inside a white square box of 63 × 63 × 63 cm^3 composed of three white foam walls and a white curtain covering the fourth side. Lighting was provided by two small halogen lights held on the lateral walls and pointing to the white fabric. Video recording was made from above, using a Sony Handycam (HDR-CX740).

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