idTracker: User Guide

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Contents

1 User guide

1.1 Quick start

This section describes the installation of the Windows stand-alone version of the program, and its use to track a video, such as the example video of five zebrafish that can be downloaded from www.idtracker.es. If you want to record your own video, first read Section 1.2.

1. **Install idTracker.** We strongly recommend to use Windows 7 (64 bits). idTracker also works under Windows XP (64 bits), but it does not recognize most video codecs. Download idTracker_setup.exe from www.idtracker.es, execute it and follow the instructions. In addition to idTracker, it will automatically install the Matlab Compiler Runtime (unless you already have it).

2. **Copy the video to an adequate location.** idTracker will generate a large amount of data (depending on the length of the video and the number of individuals; about 500 MB for the example video), so there must be free space in the disk. Also, idTracker needs to access big amounts of data frequently, so it will be extremely slow if the video is an external hard drive with a slow connection to the computer (e.g. USB 2.0). Place the video either in the internal hard-drive, or in a external hard drive with fast connection (e.g. eSATA or USB 3.0).

3. **Execute idTracker.**

4. **Open file.** The first window lets you select one file from your computer. Select the file with the video. If your video is split in several files, select the first one (see Section 1.2 for the format of videos split in several files).

5. **Main screen.** A window like the one shown in Figure S1 will open. From now on, all references to components in this main window refer to the labels in Figure S1

   If you are using the example video provided in www.idtracker.es (5Zebrafish_nocover_22min.avi), you may jump directly to step 15 and skip the adjustment of all parameters, as default values work well for this video.

6. **Enter the number of individuals** in box 4. For the example video, enter 5.

7. **Choose type of contrast.** By default, the system looks for dark animals on bright background. Check the box 'Invert contrast' (9) to change to bright animals on dark background. For the example video, leave it unchecked.

8. **Select Region Of Interest (ROI) and/or exclude regions.** If you want to select a Region of Interest, click on button 12 and follow the instructions. You can also choose a region to be excluded from the tracking with button 13. Click on button 14 to clear all previously defined ROI's or excluded regions. For the example video it is not necessary to select any ROI.

9. **Background removal option.** Check the box 8 if you want to activate the background removal option. The background will be computed when the tracking starts. To compute it before (so that you can view the correct results in the visualization window 3) click on the 'Compute Bckgrnd' button 8. It will take a few seconds, the progress is shown in one of the progress bars 18. A red '!!' on the right of the 'Compute Bckgrnd' button means that the background is not updated (meaning that the segmentation presented in panel 3 is not updated, but has no effect in the final result of the tracking). For the example video, leave 'remove background' checked and click 'Compute Bckgrnd'.

10. **Enter the intensity threshold.** Use box 5 to change the intensity threshold for segmentation. The system considers that pixels with lower intensity than this threshold belong to the animals (or higher intensity, if "invert contrast" is checked). You can check the result for each threshold in the visualization window (panel 3. Green patches indicate the segmented blobs). You can use box 19 to view different frames. For the example video, threshold 0.8 works well.

11. **Enter the minimum size.** The program will reject blobs smaller than the minimum size entered in box 6. Watch several frames (use box 19 to change the frame), and choose a value that is that is clearly smaller than the sizes of the animals (the bar 20 on the bottom shows the sizes of the animals in the current frame. Each black vertical line corresponds to one animal, and the red line is the minimum size). For the example video, minimum size 250 works well.

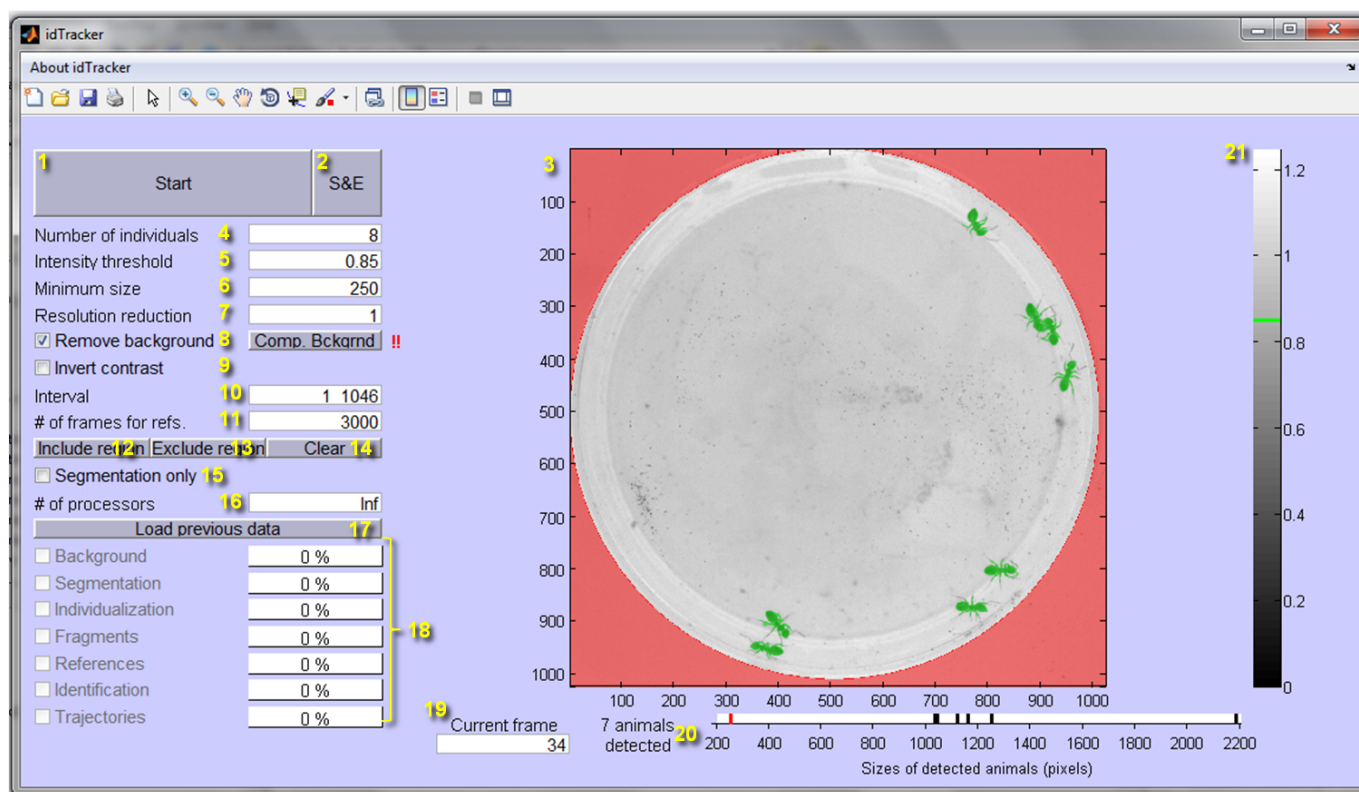12. **Choose resolution reduction.** If the size of the

Figure S1: Main interface of idTracker.

animals is very big compared to the image, the algorithm will take too long. If the sizes of the animals are bigger than 2000 pixels, input a number higher than 1 in box 7 (the number of pixels will be divided by n^2, where n is the number in box 7. Choose this number so that the resulting number of pixels of the blobs is below 2000). For the example video, leave a 1 in box 7.

13. **Choose an interval.** If you want to track only part of the video, enter the interval that you want to track in box 10 (in frames, use a space to separate the first frame and the last frame of the interval). For the example video, leave the full interval.

14. **Number of reference frames.** Box 11 shows the maximum number of reference frames that the program will use. Choose a lower number for increased speed, a higher number for increased accuracy. For the example video, leave it at 3000.

15. **Start tracking.** Click the 'start' button (1). In some computers, after pressing 'Start' the operating system's firewall will issue a warning that idTracker is trying to access the local network. This is caused by idTracker trying to connect to several processors of the computer simultaneously (it does not connect to the internet). In order to use more than one processor, grant access at this point. Once the tracking is started, you will not be able to change the parameters. Wait until the program finishes (a new window will appear indicating that the tracking has finished). It may take several hours, an in the meantime idTracker's window may not have a very smooth behavior (for example, if you minimize and maximize it again it is normal that it stays black for a long while).

16. **Get the results.** The results are in a folder called 'segm' located in the same folder as the video. The trajectories are in the Matlab file trajectories.mat (this file contains a 3D matrix called trajectories that contains the coordinates of each individual in each frame. The first dimension runs along frames, the

3

second along individuals and the third along x, y). They are also in the file trajectories.txt in text format (in this file, each two columns correspond to the x, y coordinates of each individual.

1.2 Conditions for the video

idTracker needs the following conditions to work:

- **Background.** Ideally, there should be a uniform background, with a color that gives enough contrast with the animals. Different degrees of background structure are acceptable, and will be managed by the system in different ways:

  - Static or moving objects much smaller than the animals will not affect segmentation, because objects below a certain size are removed.

  - Static objects of any size can be removed in two alternative ways: They can be manually excluded when selecting the region of interest, or they can be automatically excluded by the background removal option. In either case, when an animal is in contact with one of these objects its segmentation is less consistent, and the segmented image is not used for identification. The animal can still be identified with images of the neighbouring frames (see section **??**), but the performance of the tracking may be lower for the portions of the video in which the animals overlap with the background objects.

  - Static or moving objects of any size that are present only in a small portion of the video may cause problems in the identifications of animals while present, but will not affect the rest of the video (see comments about robustness to perturbations in the main text).

- **Camera.** The system works on videos recorded with conventional cameras, including consumer handicams and even some modern photo cameras. The system works on grayscale images, so if the video is in color it will first transform it to grayscale. General requirements are the following.

  - Resolution of the video must be enough for identifications, meaning that the images of the animals must have a minimum size (in pixels). We have performed tracking successfully with as few as 150 pixels per animal (area of the segmented blobs, average across all individuals and full video), and in our experience 250 pixels per animal is enough for most species and conditions. See Table S1 for the resolution conditions of the videos used for validation.

  - Frame rate should be high enough so that the images of an animal moving at typical speed overlap in consecutive frames. We find that 25 fps is enough for all species used in the validation.

  - Exposure time should be short enough with respect to the typical speed of the animals, so that images are not blurred (a small proportion of burred images is acceptable, for example when animals turn very suddenly).

  - If the video is compressed, it should be high quality and high definition compression (for example we find good results with codecs AVHCD or HD mpeg4, which are typical in most modern consumer cameras). The system may sometimes work with medium-quality compression, but at the expense of having higher resolution (relative to body size) than would be required with uncompressed video.

- **Video format** must be compatible with Matlab, which can only access some of the codecs installed in the computer. Compatible formats may be different on different computers (especially if they have different operating systems). In Windows computers, we find that uncompressed avi or mpeg4-compressed avi files are usually readable. See www.idtracker.es for more information about converting videos to compatible formats. If the video is cut into several files, the last characters of the file names must be the number of the file, with no trailing zeros. The rest of the file name must be identical for all files (for example file1.avi, file2.avi, ... file9.avi, file10.avi ...).

- **Arena.** Ideally, the walls of the arena should be transparent or translucid, so that there are no strong shadows near the walls (sometimes opaque walls work fine when illumination is very diffuse). Reflections of the animals on the walls can be a problem, but usually they can be removed by selecting a tight Region of Interest that removes the walls. For aquatic animals with transparent walls, having water at both sides of the wall helps to soften the reflections. If a

cover is needed to prevent the animals from escaping, it should be as transparent, and clean as possible. Care must be taken to prevent intense reflections from the cover (or from the water surface in the case of aquatic animals). See the description of the set-up in section 2.1 for an example of how to prevent reflections.

- **The apparent size of each individual should not change very much during the video.** Therefore, the following conditions should be satisfied.

  - To prevent large changes of distance between camera and animals, the animals should move roughly in 2D. Animals that walk on a plane automatically obey this restriction. Swimming or flying animals should be restricted so that they move in a narrow region, which can be wide enough to allow crossings (this region can be as thick as 2 to 5% the distance between the camera and the animals).

  - The camera should point perpendicularly to the plane in which the animals move. This it to prevent perspective effects that change the apparent size of the animals when they change position.

- **Illumination** should be as uniform as possible. Indirect illumination usually gives the best results. Retroillumination is also an option, and we have successfully tracked retroilluminated videos (in our case there was also some ambient light, so the animals' skin patterns were still visible. When the patterns are not visible the identification is more difficult, but it may still work fine for some species and conditions).

- **Shadows** of the animals on the background may affect the segmentation, making the identifications more difficult. These shadows can be prevented by using a transparent base for the set-up, with the opaque background at a certain distance behind the animals (see for example the set-up described in section 2.1). In this way, the shadows on the opaque background are very diffuse and do not affect segmentation.

- **Duration of video.** Collection of references requires a minimum length of the video in which all the animals are in view. This length depends on the number of animals, the species and their level of activity. As a reference, for groups up to 10 zebrafish usually 5 minutes is enough. 30 minutes is a safe value (we have never found a 30-minutes video in which the system fails to collect references). If the video is too short or the animals are not visible for a long time, the video can still be tracked using external references.

1.3 Description of the interface

The interface has the following controls (numbers correspond to those in Figure S1).

1. **Start button.** When pressing this button the tracking process starts, and tracking parameters cannot be changed any more.

2. **Save & Exit button.** When pressing this button the program ends, but the tracking does not start. All tracking parameters are stored in file datosegm, so they can be used at a later time using button 17.

3. **Main visualization window.** This window shows a frame of the video (use box 19 to select the frame), with the current output of segmentation. It is updated whenever any parameter is changed. You can zoom in and out in this window with the tools provided in the toolbar. Red regions are excluded from the tracking, either because they are outside the region of interest (button 12) or because they have been excluded (button 13). Green regions are the blobs that have been segmented in the current frame.

4. **Number of individuals.** Edit this box to input the number of individuals that are present in the video. Not all individuals need to be visible at all times, this must be the total number of individuals.

5. **Threshold for segmentation.** Only pixels darker (i.e. with lower value) than this threshold will pass segmentation (see also point 9, 'invert contrast'). The value of the threshold is given as proportion of the average intensity of the whole frame.

6. **Minimum size.** Only blobs bigger than this size will be selected.

7. **Resolution reduction.** Resolution can be reduced to speed up the first stages of the algorithm. It is strongly recommended to reduce resolution if blobs are bigger than 5000 pixels. The width and height of each frame is divided by the number in this box (i.e. 1 means the original frame, 2 means that width and height will be reduced to one-half, so the total amount of pixels will be reduced by 4 times).

8. **Remove background.** Check this box to activate a routine that will prevent static objects from being confused with animals. When the checkbox is activated, the button 'Comp bckgrnd' appears. Push this button to compute the background with the current settings. Before the background is computed (or if any parameter that affects background calculation changes) a red '!!' sign will appear. This sign means that the output shown in the main visualization window (window 3) is not accurate because it has not been computed using the true background. But this sign only refers to visualization, if the button 'Comp bckgrnd' is not pressed (or any parameters change and it is not pressed again), the background will be computed after pressing 'Start' (button 1).

9. **Invert contrast.** By default, the program looks for dark animals on a bright background. When this box is checked the program looks for bright animals on dark background.

10. **Interval.** This box indicates the interval of video (in frames) that will be tracked. By default indicates the full video.

11. **Number of frames for references.** Maximum number of images of each individual that will be incorporated in the references. The program will acquire as many reference images as it can, up to this number. A lower number will make the process faster and will use less memory, at the expense of higher error rates (for many species and situations about 500 frames in the references can be used without appreciable increase in error rates, but if computation time and memory are not pressing constraints, we recommend to leave it at the default value of 3000).

12. **Region of Interest.** Press this button to select a region of interest, or to expand it once it has been created[1]. A new dialog will appear, giving choice between rectangular, circular or polygonal region. Once the selection is made, go to the main visualization window (window 3), and select as many points as necessary, depending on the type of region:

- For rectangular region select 2 points, which will be opposite corners of the rectangle.
- For circular region select 4 points. The border of the region will be the circle that best fits these 4 points.
- For polygonal region select a minimum of 3 points, with no upper limit. Each point will be a corner of the polygon, and it is not necessary to finish on the same point as you started, a last side will be added between the last and the first points. Press ENTER once all corners have been selected. The polygon does not need to be convex.

13. **Exclude region.** Press this button to exclude a region. You can select a region with the same three geometries as for the region of interest. Excluding a region will not erase a previous region of interest, nor previous excluded regions. It is not necessary to have selected a region of interest to exclude a region.

14. **Clear region of interest and excluded regions.** Press this button to remove the region of interest and all excluded regions.

15. **Segmentation only.** If this box is checked, idTracker will exit after the segmentation step, leaving the tracking unfinished. The data can be recovered later using button 17.

16. **Number of processors.** IdTracker can use multiple processors to improve performance. The number of this box indicates how many processors idTracker will use ('Inf' means that idTracker will use all available processors). Using a lower number of processors leaves more resources for other programs while idTracker is running. Memory usage is lower when using fewer processors, so using fewer processors may be necessary in computers with low RAM memory, especially when tracking videos with many individuals.

17. **Load previous data.** This button will only be active if the program has been executed previously on the same video (even if the tracking process has not finished). When pressing it, all current parameters are erased and substituted by the parameters in the previous run. Also, all intermediate steps that were completed in the previous run (background, segmentation, etc.) will be recovered and can be re-used.

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[1] The behavior of this button changes once a ROI has been defined. At the beginning (when the button reads 'ROI') the region selected with this button will be the region of interest, and all pixels outside it will be excluded. But as soon as some ROI or excluded regions have been selected the behavior of this button changes to 'include region', and any region selected with this button will be added to the current ROI.

18. **Process indicators.** These bars measure the process of each stage of the tracking. When a previous run of the tracking has been performed and the data have been loaded (using button 17), the user can choose whether to reuse different parts or not. Check the boxes to reuse parts, un-check them to recompute them (note that recomputing one part implies that all the parts that come afterwards will also need to be recomputed).

19. **Frame shown.** This box controls what frame is shown in the main visualization window (window 3).

20. **Summary of blobs detected.** It indicates how many blobs have been segmented in the current frame, and their sizes (black lines). The red line indicates the minimum size that has been specified in box 6.

21. **Colorbar.** It shows the grayscale used to show the frame in the main visualization window (window 3). The green line indicates the threshold for segmentation (introduced in box 5).

2 Description of the set-ups

This section describes the set-ups 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 section 1.2 for a general description of the conditions required).

All videos have been recorded with a monochrome Basler A622f camera that has a resolution of 1280×1024 pixels. It is connected to a computer via Firewire (IEEE 1394), and at full resolution has a frame rate around 25 fps (the frame rate increases when we decrease resolution using only one part of the camera's sensor. We typically select a Region of Interest that fits tightly on the arena, so most videos are recorded at higher frame rates, see Table S1). The videos are directly recorded on the computer's hard disk, their length being limited only by the hard disk capacity (around 20 hours of uncompressed video in a 2 TB hard disk). The rest of the set-up is as described below for each species.

2.1 Fish from above (I)

We have used different setups to record fish from above. The main difference is that for some videos we place 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). This section describes the setup with a transparent cover (see section 2.2 for set-up without roof).

The set-up for part of the videos of zebrafish (*D. rerio*), nine-spined stickleback (*P. pungitius*) and Medaka (*O. latipes*)is a $50 \times 50 \times 2$ cm (length $\times$ width $\times$ 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 is completely closed including a transparent roof (but not watertight), and fully submersed into a larger tank ($90 \times 120 \times 20$ cm) equipped with a water acclimatizer to maintain healthy conditions for the fish. The arena is sustained by four legs at around 5 cm above the white floor of the larger tank, so that the shadows of the fish are diffused, facilitating the segmentation. The camera is situated over the set-up 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 set-up covers around 950×950 pixels in the image. In order to have indirect and uniform illumination, we use six halogen floodlights (500 W each) pointing to the ceiling. A brown surface of 120×150 cm at the level of the camera prevents the light directly reflected on the ceiling to reach the set-up, so that illumination reaching the setup is indirect. Also, this brown surface projects a dim and uniform reflection on the water surface.

2.2 Fish from above (II)

The set-up for part of the videos of zebrafish (*D. rerio*) and Medaka (*O. latipes*), is a $62 \times 45 \times 18$ cm (length $\times$ width $\times$ height) plastic box, filled with water up to 3 cm and surrounded by water that is circulating through an acclimatizer. This box has no roof. The walls and floor of the box are translucid, and the box is at around 10 cm above the white floor of the larger tank. Illumination and camera disposition are the same as for the previous set-up. In this case, the set-up covers around 1150×810 pixels in the image.

2.3 Juvenile fish

The set-up for juvenile *nacre* zebrafish (*D. rerio*) is a Petri dish of diameter 8.5 cm. The dish is covered by a transparent perpex lid, and fully submerged in water. We place a black plastic below the dish, because we found that nacre zebrafish have better contrast in this way. Illumination and camera are the same as for the previous cases, but with the camera closer to the set-up.

2.4 Fish from the side

The set-up to record zebrafish from the side is a $25 \times 3 \times 25$ cm (length $\times$ width $\times$ height) chamber inside a bigger tank made of glass. The Basler A622f camera is at 1 m from the set-up, pointing horizontally towards the 25×25 cm face of the set-up. Illumination is the same as for our fish-from-above set-up. Black background gives the best contrast in this case, so we place a black curtain over the rear side of the tank. We also place a black curtain around the camera's objective, in order to obtain a dim and uniform reflection from the glass tank.

2.5 Mice (*M. musculus*)

The video with 4 mice was recorded inside a transparent plastic cage of size $30 \times 47 \times 35$ cm (length $\times$ width $\times$ height). The walls are high enough to prevent the mice

from escaping, so the cage is open at the top. Videos with 2 mice were recorded in a transparent plastic cage of size $18 \times 32 \times 20$ cm, covered with a transparent 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 identical to our fish-from-above set-up, with the camera at around 110 cm and 100 cm from the floor of the set-up for the 4-mice and 2-mice videos, respectively.

2.6   Insects

Flies (*D. melanogaster*) and ants (unknown species, collected from a park in Madrid, Spain) were recorded in a circular arena of diameter 5.5 cm. The floor of the arena is made of transparent perspex. Walls and roof consisted of a Petri dish 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 to climb to the walls and roof. With this configuration we could record walking flies with no need to cut their wings. The Basler A622f camera was placed 10 cm below the set-up, pointing upwards. The objective used in the other conditions cannot focus at such a short distance, so this time we used a Pentax C31635KP - C1614-5M (KP) (focal length 16 mm). The insects were therefore seen through the transparent floor, against the white background of the petri dish covered with Fluon. The set-up was surrounded by white curtains, and illumination was provided by five hallogen floodlights (500 W each) outside the curtains, and pointing towards the inside.

3 Validation

We performed validation on several videos of different species, summarized in Table S1 and in Figures S2-S4.

The process of validation of each video is as follows. First, we ran idTracker on the whole video. Then, we reviewed manually each crossing of a portion of the video[2]. We chose the initial portion, and corrected the identities when necessary. Figures S1-S3 give an analysis of the validation results for several species. These figures contain several boxes for each video: Box (a) gives a representative frame of the video; (b) gives a histogram of the sizes of the individuals; (c) gives the correctly labeled trajectories in color, and the incorrect portions in black.

To identify an animal, the system uses all the frames between two consecutive crossings (all the frames of a fragment, see section **??**). Mistakes are less likely when the animal travels a longer distance between consecutive crossings, so we analyzed the probability of mistake as a function of this distance (Figures S2-S4, box d). Each blob that corresponds to a single individual may be in one of these three states: Identified with the correct identity (green), not identified (orange) or identified with the wrong identity (red). In all cases we find that the probability of mistake is low even for the shortest fragments, and decreases fast as the length of fragments increases (most mistakes occur in fragments shorter than 1 body-length, and we never found any mistake for fragments longer than 5 body-lengths). Short fragments make up a very small proportion of the video, so overall proportion of mistakes is extremely low. All videos have more than 97 % of the trajectories correctly identified, and most of them more than 99 % (Table S1).

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[2]It is too time-consuming to review the whole videos. Because the performance of idTracker does not deteriorate with time, any portion of the video is representative of the whole sequence.

Table S1: **List of videos used for validation.** To validate the system, we reviewed manually every crossing in a representative portion of each video. 'Time reviewed' indicates the length of the portion that is reviewed and used for validation. 'Revision time factor' indicates how many minutes were needed to review each minute of video (i.e. the product of 'Time reviewed' times 'Revision time factor' gives the time that it took to review the portion of the video). 'Area of animals' is the average area of the segmented blobs that are classified as individual animals. '% frames correct' indicates the percentage of blobs that were correctly identified (i.e. $100\ n_{\text{correct}}/(n_{\text{correct}} + n_{\text{incorrect}} + n_{\text{not identified}})$, where the parameters n stand for number of blobs of single individuals with correct identities, incorrect identities and not identified, respectively). '% distance correct' indicates the percentage of the length of the trajectories with correct identities. 'Section describing set-up' indicates the section of this document that describes the set-up where each video was recorded.

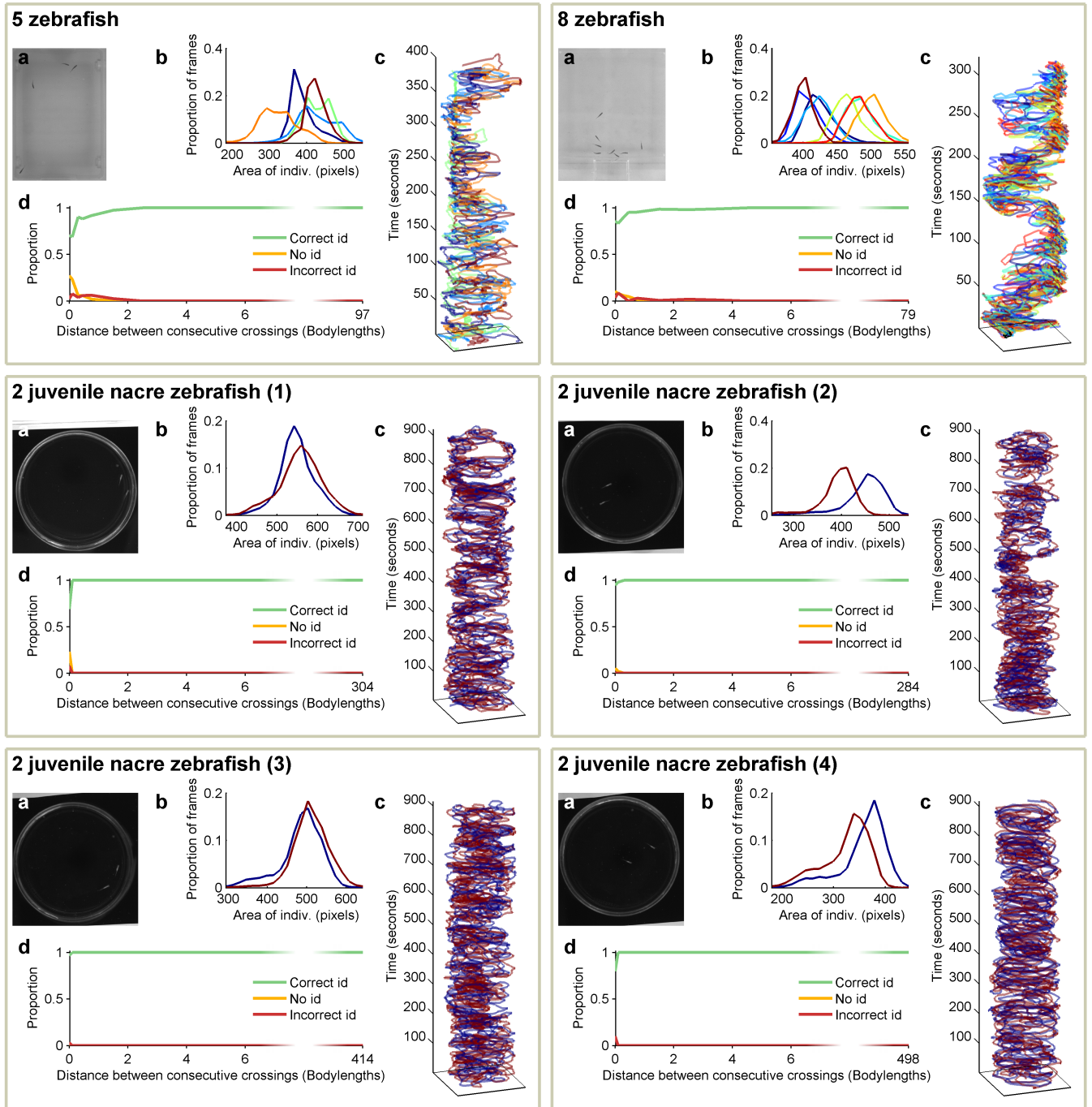| Video	Length of video (min)	Frame rate (fps)	Section describing set-up	Area of animals (pixels)	Time reviewed (min)	Revision time factor	% frames correct	% distance correct
5 medaka fish	32.1	28	2.1	210	17.9	0.3	99.99	>99.99
10 medaka fish	32.0	28	2.1	153	6.2	4.7	99.91	99.96
20 medaka fish	60.3	29	2.1	226	5.7	5.2	99.95	99.98
8 ants	32.7	28	2.6	804	3.0	-	91.91	99.14
8 zebrafish	5.4	28	2.1	448	5.4	52.1	99.56	99.67
5 zebrafish without cover	30.5	32	2.2	397	5.2	7.7	99.50	99.77
2 juvenile nacre zebrafish (1)	15.3	25	2.3	553	15.3	-	99.99	>99.99
2 juvenile nacre zebrafish (2)	15.3	25	2.3	419	15.3	-	99.99	99.99
2 juvenile nacre zebrafish (3)	15.3	25	2.3	497	15.3	0.5	99.99	>99.99
2 juvenile nacre zebrafish (4)	15.3	25	2.3	340	15.3	0.3	99.99	>99.99
8 drosophila flies	32.3	28	2.6	762	2.4	-	99.17	99.91
2 agouti mice	31.5	49	2.5	11873	3.4	-	98.73	99.09
2 black mice (1)	31.5	49	2.5	12394	10.2	3.1	97.20	97.76
2 black mice (2)	31.4	49	2.5	11756	3.4	2.6	98.20	98.36
2 black mice (3)	31.5	49	2.5	12740	3.4	2.1	98.07	98.98
4 black mice	31.5	25	2.5	8968	6.7	4.5	98.06	98.98

Figure S2: **Results of validation for zebrafish.** For each video: **(a)** Example frame. **(b)** Distribution of sizes (area of the segmented blobs) of each individual along the validated portion of the video. **(c)** Trajectories (x and y axes correspond to the sides of the set-up, z axis shows time). Colors represent correct identities, and black represents wrong identities. **(d)** Probability of outcome for single-individual fragments of different lengths. Green for correct identity, orange for non-identified fragment, and red for wrong identity. The x-axis extends up to the longest validated fragment.
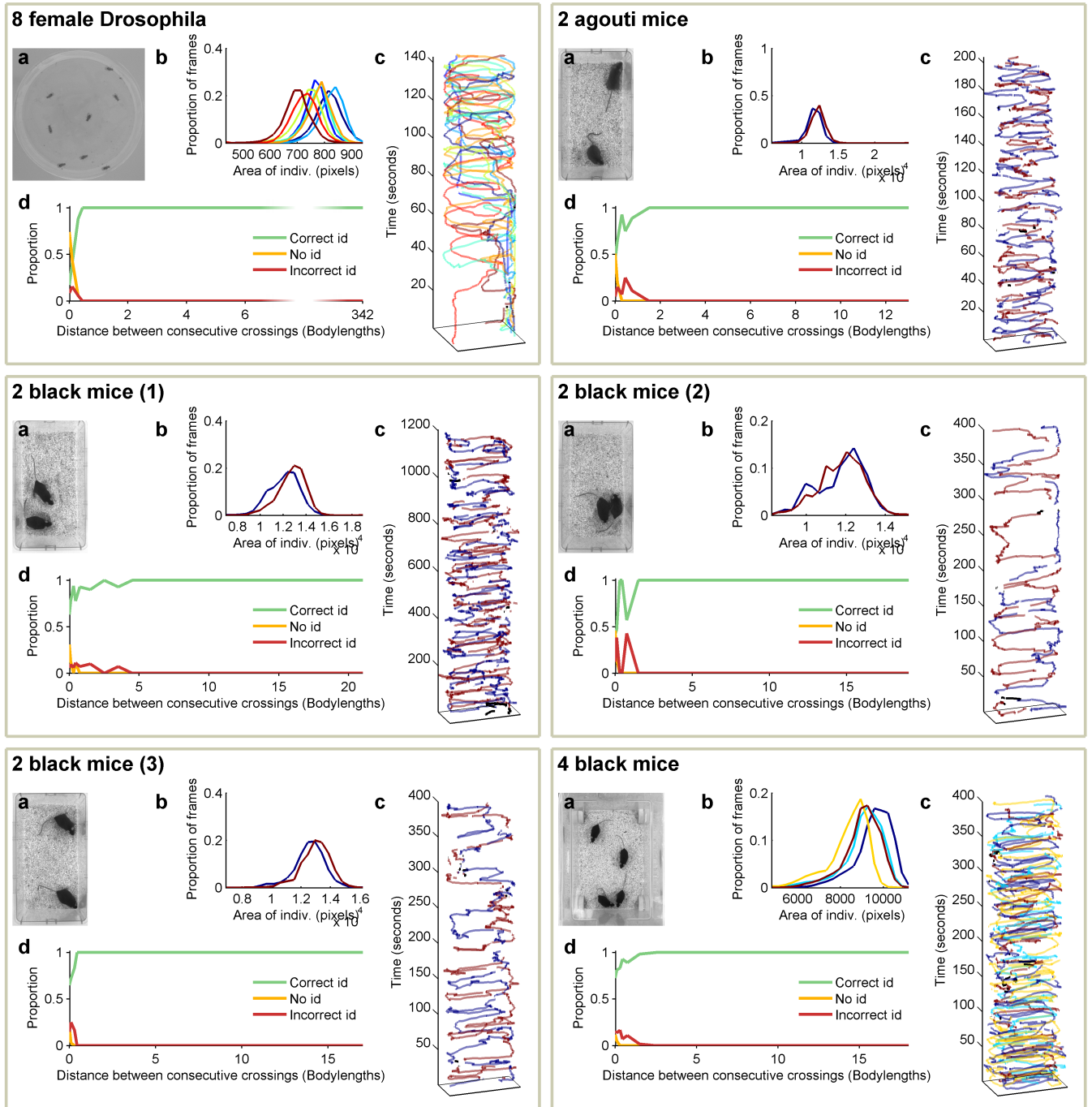
8 female Drosophila

2 agouti mice

2 black mice (1)

2 black mice (2)

2 black mice (3)

4 black mice

Figure S3: **Results of validation for Drosophila and mice.** For each video: **(a)** Example frame. **(b)** Distribution of sizes (area of the segmented blobs) of each individual along the validated portion of the video. **(c)** Trajectories (x and y axes correspond to the sides of the set-up, z axis shows time). Colors represent correct identities, and black represents wrong identities. **(d)** Probability of outcome for single-individual fragments of different lengths. Green for correct identity, orange for non-identified fragment, and red for wrong identity. The x-axis extends up to the longest validated fragment.
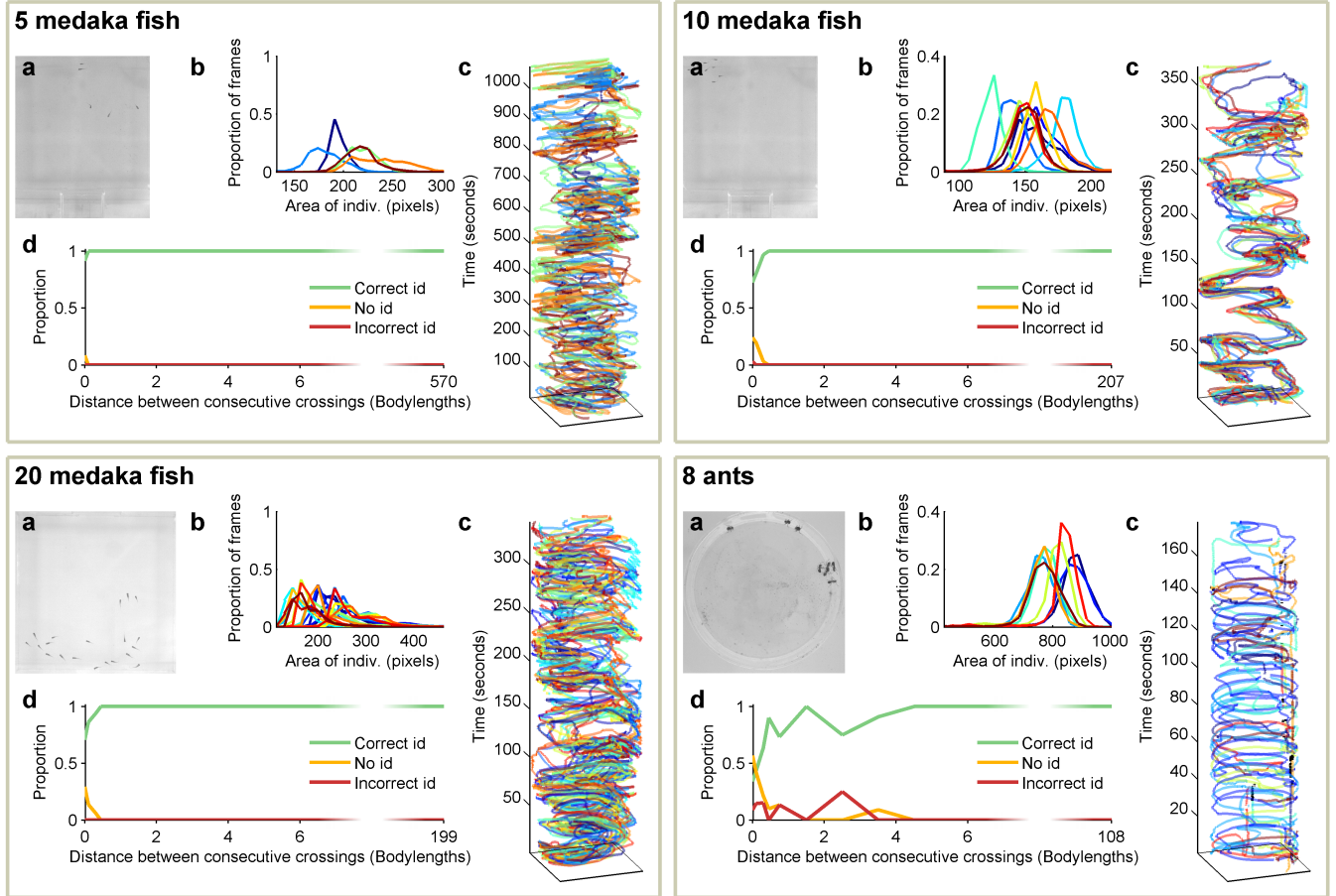
13

5 medaka fish

a

b

c

d

10 medaka fish

a

b

c

d

20 medaka fish

a

b

c

d

8 ants

a

b

c

d

Figure S4: **Results of validation for medaka and ants.** For each video: **(a)** Example frame. **(b)** Distribution of sizes (area of the segmented blobs) of each individual along the validated portion of the video. **(c)** Trajectories (x and y axes correspond to the sides of the set-up, z axis shows time). Colors represent correct identities, and black represents wrong identities. **(d)** Probability of outcome for single-individual fragments of different lengths. Green for correct identity, orange for non-identified fragment, and red for wrong identity. The x-axis extends up to the longest validated fragment.