Supplementary Material

Automated image-based tracking and its application in ecology

Anthony I. Dell*, John A. Bender, Kristin Branson, Iain D. Couzin, Gonzalo G. de Polavieja, Lucas P.J.J. Noldus, Alfonso Pérez-Escudero, Pietro Perona, Andrew D. Straw, Martin Wikelski, and Ulrich Brose

*Corresponding author: adell@gwdg.de

Table S1. Some key automated image-based tracking systems (in alphabetic order) that are readily available to research ecologists, with focus on those suited for tracking behavior during species interactions. See Table S2 for detailed descriptions of each category.

3DTracker [1] (Movie S22)

Availability and usability: open-source, simple to use, specific[1]
Raw output: 3D trajectories, 3D pose[2]
Requirements: Win, Matlab, multiple 3D cameras[3]
Maximum individuals: 2
Shape requirements: rodent-like[4]
Identity method: crosses solved[5]
Analysis tools: basic kinematics, social behaviour[6]
Taxa studied: rats[7]
Required resolution per individual: 300 points[8]

Notes: [1]Executables and sources available online at http://matsumotoj.github.io/ [1]. Requires uncluttered background. Requires multiple 3D cameras. [2]Estimates 3D trajectories and detailed 3D pose of four body parts (head, neck, trunk, and hip). [3]Currently uses 4 x Microsoft Kinect cameras. Matlab required for behavioral analysis. [4]Developed for rats, but could be applied to mice with higher resolution cameras. [5]3D video enables more stable tracking during close contact. On average, automation makes errors in 20% of 1 min videos. Includes tool to facilitate manual correction. [6]Includes Matlab scripts extracting basic movement parameters (such as velocity or angle) and social behaviors (e.g., approaching, mounting, head-hip contact) based on 3D trajectories of body parts. [7]Social interactions in rats [1]. [8]3D points covering the surface of each individual.

CADABRA [2] (Movie S14)

Availability and usability: open-source, simple to use, flexible[1]
Raw output: 2D trajectories, detailed pose[2]
Requirements: Win/Linux/Mac, Matlab[3]
Maximum individuals: 2
Shape requirements: fly-shaped[4]
Identity method: maintained (size/markings)[5]
Analysis tools: extensive[6]
Taxa studied: flies[7]
Required resolution per individual: 20 pixels

Notes: [1]Executables available online [2]. Source code available upon request. Flexible to different setups. Can use conventional cameras. Can track several arenas simultaneously. Requires constant and uncluttered background. [2]Estimates 25 traits of the pose and position of each individual (orientation, velocity, size, wing pose). [3]Matlab Compiler Runtime library (free). [4]Developed for *Drosophila*. [5]Uses size difference between pairs (e.g., male or female). Similar sized individuals must be marked (otherwise system makes highest probability matches). [6]Uses estimates of 25 traits of both flies (position, orientation, velocity, size, wing pose) to automatically quantify 8 social behavior (e.g., lunging, wing extension, copulation). [7]Genetic and environmental influences on social behavior in *Drosophila* [2, 3].

Ctrax [4] (Movie S1, Movie S3)

Availability and usability: open-source, simple to use, flexible[1]
Raw output: 2D trajectories, orientations
Requirements: Win/Linux/Mac, Matlab[2]
Maximum individuals: 50
Shape requirements: elliptical[3]
Identity method: crosses solved[4]
Analysis tools: extensive, see JAABA[5]
Taxa studied: flies, cockroaches, fish, spiders[6]
Required resolution per individual: 15 pixels

Notes: [1]Ctrax online at http://ctrax.sourceforge.net. Flexible to different setups. Can use conventional cameras. Requires constant and uncluttered background. Ongoing maintenance, enhancements, and support provided. [2]Matlab required for capabilities beyond tracking, which are important (i.e., error fixing, analysis). [3]Developed for *Drosophila*, works well with any species of elliptical shape. Individuals must have similar size. [4]For *Drosophila*, with full automation system makes an identity error on average once every 5 fly-hours with density of 10, once every 1.5 fly-hours with dentisy of 20, and once every 40 fly-minutes with density of 50. Error rate is zero with minimal user supervision. Includes application to facilitate manual correction. [5]JAABA [5] supersedes Ctrax's behavioral analysis package (free online at http://jaaba.sourceforge.net). JAABA is a machine-learning system to create automatic behavior classifiers, which allows exploration of differences in thousands of behavior statistics between large numbers of individuals. JABBA requires tracking data from other tracking systems (e.g., Ctrax, CADABRA, Multi-Worm Tracker, Motr). [6]Genetic and environmental influences on *Drosophila* social behavior [4, 6], cockroach locomotion [7], collective behavior in fish [8], spider foraging behavior (Dell et al. unpublished).

EthoVision XT (Movie S4)

Availability and usability: commercial, simple to use, flexible[1]
Raw output: 2D trajectories, orientations[2]
Requirements: Win
Maximum individuals: 16[(note 3)]
Shape requirements: flexible[4]
Identity method: maintained (size/markings)[5]
Analysis tools: extensive[6]
Taxa studied: rodents, fish, insects, pigs, spiders, more[7]
Required resolution per individual: 15 pixels[8]

Notes: [1]Developed by Noldus Information Technology BV. Available online at http://www.noldus.com/ethovision. Free trial version available. Software only or with integrated hardware. Valid for conventional cameras and laboratory setups, including multiple arenas. A constant and uncluttered background not required. Shadows, bedding material and objects can be present in the arena. Dedicated systems available for fish (DanioVision) and rodents (PhenoTyper). [2]Track3D (http://www.noldus.com/innovationworks/products/track3d) extension allows 3D tracking of single individuals using 2 cameras. Standard software tracks center of body mass, specialized modules recognize body shape and multiple body points (e.g. nose point, tail base) of rodents. Simple and easy-to-use interface. [3]Can track 100 arenas (16 individuals in each arena) with a single camera.) [4]Can track many shapes, from small insects to large mammals. [5]Similar-sized individuals require color marking (when maintenance of identity required). With small numbers of individuals the system can often maintain identities of unmarked animals. [6]Includes visualization, data editing and animation options. Module for automated behavior recognition (rodents: rearing, grooming, sniffing), trial and hardware control, behavior-physiology integration. [7]See all publications at http://www.noldus.com/ethovision-xt/selected-publications. [8]Requires 3 pixels per individual for position only.

Flydra [9] (Movie S6)

Availability and usability: open-source, difficult to use, specific[1]
Raw output: 3D trajectories (real-time)
Requirements: Linux, multiple cameras
Maximum individuals: 3[(note 2)]
Shape requirements: flexible[3]
Identity method: not maintained
Analysis tools: none
Taxa studied: flies, birds[4]
Required resolution per individual: 1 pixel

Notes: [1]Core 3D algorithms available under open-source license at https://github.com/strawlab/pymvg (image acquisition and synchronization code, extended kalman filter for tracking, and analysis tools to verify tracking is working are not currently open-source). Requires synchronization of multiple computers and high-speed cameras. Requires constant and uncluttered background (some degree of habitat complexity possible). Advanced Linux skills needed. [2]Limited by computer speed. [3]Individuals should be roughly the same size, and have independent behavior (i.e. not valid for social species, aggression, courtship, predation, etc.). [4]Flight control in *Drosophila* and hummingbirds [9-13].

GroupHousedScan (Movie S15)

Availability and usability: commercial, simple to use, flexible[1]
Raw output: 3D trajectories, detailed pose (real-time)[2]
Requirements: Win, two cameras[3]
Maximum individuals: 2[(note 4)]
Shape requirements: rodent-like[5]
Identity method: maintained (size/markings)
Analysis tools: extensive[6]
Taxa studied: rats, mice, primates[7]
Required resolution per individual: 200 pixels[8]

Notes: [1]Developed by CleverSys Inc. Available online at http://cleversysinc.com/?csi_products=grouphousedscan. Software only or with integrated hardware. Valid for conventional cameras and laboratory setups. Requires contrasting background. Ability to easily characterize spatial components of the physical environment for integration with tracking data. Bedding material, food and water containers, and other objects can be present in the arena. Useful for long-term tracking over longer durations, such as multiple days, as is capable of adjusting between day and night conditions automatically. Simple and easy-to-use interface. [2]Dedicated 2D systems can monitor the movement and detailed pose of single animals either from the side (HomeCageScan - http://cleversysinc.com/?csi_products=homecagescan and PrimateScan - http://cleversysinc.com/?csi_products=primatescan) or top (TopScan - http://cleversysinc.com/?csi_products=topscan-suite). TopScan can be extended to 4 individuals with SocialScan add-on. Pose data include 8 different body points on a rodent: head or nose, ears, forelimbs, hindlimbs, upper back, lower back, abdomen, tail. HomeCageScan identifies same 8 points. PrimateScan identies same 8 points on a primate. TopScan and SocialScan identify 4 points on a rodent: nose, forelimb, center of mass, tailbase. [3]Single-camera options available (see note 2). [4]see note 2. [5]Developed for mice and rats, has dedicated system for a single primate (see note 2). [6]Kinematic measurements such as speed, velocity, orientation, shape, etc. Automatically outputs individual (eat, drink, sleep, walk, jump, rear up, hang, groom, sniff, twitch, stretch, etc.) and social interaction (contact, sniff, follow, leave, approach, etc.) behaviors and events. [7]Genetic and environmental disorders in rats and mice (unpublished). [8]Can be set as a threshold.

GroupScan (Movie S15)

Availability and usability: commercial, simple to use, flexible[1]
Raw output: 2D trajectories
Requirements: Win
Maximum individuals: 100[(note 2)]
Shape requirements: flexible[3]
Identity method: not maintained
Analysis tools: basic kinematics[4]
Taxa studied: *Drosophila*, fish[5]
Required resolution per individual: 10 pixels[6]

Notes: [1]Developed by CleverSys Inc. Available online at http://cleversysinc.com/?csi_products=groupscan. Software only or with integrated hardware. Valid for conventional cameras and laboratory setups. Requires contrasting background. Ability to easily characterize spatial components of the physical environment for integration with tracking data. Simple and easy-to-use interface. [2]Preset at 100, but can be varied. [3]Developed for fruit flies and fish, but extendible to any species. [4]Basic population-level statistics, including count, and average distance travelled, velocity, inter-frame body pixel change, etc. [5]Genetic and environmental disorders in *Drosophila* and fish (unpublished). [6]Can be set as a threshold.

idTracker [14] (Movie S5)

Availability and usability: open-source, simple to use, flexible[1]
Raw output: 2D trajectories, orientations
Requirements: Win/Linux/Mac, matlab[2]
Maximum individuals: 20[(note 3)]
Shape requirements: flexible[4]
Identity method: maintained (fingerprinting)[5]
Analysis tools: extensive, see idSocial[6]
Taxa studied: mice, fish, flies, ants[7]
Required resolution per individual: 150 pixels

Notes: [1]Available at www.idtracker.es. Simple to use. Adaptable to different laboratory setups. Requires image with good contrast between animals and background. Valid for conventional cameras. Can track compressed videos. [2]Development version requires Matlab (free compiled binaries do not). Fingerprinting computationally heavy, runs on conventional computer with at least 4GB RAM . [3]Depends on species and conditions. [4]No inherent contour. Can track wide range of size and shaped individuals. [5]Maintains identities automatically in videos of any length, enabling tracking with complex crossings, occlusions, or perturbations. Ability to identify individuals across videos. [6]idSocial (R. Hinz, unpublished) is an accompanying open source analysis package giving mean distances, distribution of distances, kinematic parameters of interactions, probabilities for relative distances, aggression leadership or followership hierarchies and collective behavior. [7]Movement and collective behavior in fish, flies, mice, and ants [14].

LoliTrack (Movie S19)

Availability and usability: commercial, simple to use, flexible[1]
Raw output: 2D trajectories, simple pose[2]
Requirements: Win
Maximum individuals: 24
Shape requirements: flexible[3]
Identity method: crosses solved[4]
Analysis tools: basic kinematics[5]
Taxa studied: fish, rodents, birds, insects, crustaceans, more[6]
Required resolution per individual: 9 pixels

Notes: [1]Developed by Loligo Systems. Available online at http://www.loligosystems.com/?action=shop_show&varenr=AB10190. Free trial version available. Valid for conventional cameras and laboratory setups, including multiple arenas. Software only or with integrated hardware. Does not require constant and uncluttered background. Ability to easily characterize spatial components of the physical environment for integration with tracking data. Simple and easy-to-use interface. Shuttlesoft (http://www.loligosystems.com/?action=shop_show&varenr=AB10202) is a dedicated multi-chamber system for analysis of preference or avoidance to environmental drivers, such as temperature or dissolved compounds. [2]Three points along body axis. [3]No inherent contour. Can track wide range of size and shaped individuals. [4]Error rate data not available. [5]Basic measurements per individual, such as body velocity, distance moved, turning rate, time spent in zone. Dedicated solutions avaliable upon request. [6]See all publications at http://www.loligosystems.com/index.php?action=references_show_all&menu=14&keyword=VIDEO%20TRACKING.

MiceProfiler [15] (Movie S11)

Availability and usability: open-source, simple to use, flexible[1]
Raw output: 2D trajectory, detailed pose
Requirements: Win/Linux/Mac
Maximum individuals: 2[(note 2)]
Shape requirements: rodent-like[3]
Identity method: crosses solved[4]
Analysis tools: extensive[5]
Taxa studied: rodents[6]
Required resolution per individual: 350 pixels

Notes: [1]Available online at http://icy.bioimageanalysis.org/plugin/Mice_Profiler_Tracker. Easy to use and adaptable to different laboratory setups. Requires unchanging and uncluttered background. Tracker struggles when individuals are in very close contact. [2]Behavioral analysis limited to two individuals. [3]Developed for mice. [4]Switches identities on average twice per minute. Includes tool to facilitate manual correction. [5]System includes a behavioral chronogram generator (e.g., contact events, sniffing, chase, escape) and a temporal behavioral analysis module. [6]Social interactions in mice [15, 16], rats, guinea pigs (unpublished).

Motr [17] (Movie S16)

Availability and usability: open-source, simple to use, flexible[1]
Raw output: 2D trajectory, simple pose[2]
Requirements: Win/Linux/Mac, Matlab[3]
Maximum individuals: 6[(note 4)]
Shape requirements: elliptical[5]
Identity method: maintained (marking)[6]
Analysis tools: extensive, see JAABA[7]
Taxa studied: mice[8]
Required resolution per individual: 5000 pixels[9]

Notes: [1]Available at http://motr.janelia.org. Can use conventional cameras. Requires unchanging and uncluttered background and uniform illumination. [2]Pose represented as an ellipse, including x,y coordinates, major and minor axis of ellipse, and ellipse orientation. [3]Matlab 2009b or later. [4]System validated up to 6 individuals, possibly can manage more. [5]Developed for rodents, but generalizable to other elliptical-shaped organisms. [6]Requires that individuals are marked – might also work with natural differences in pattern, but this has not been tested. The system automatically learns external appearance of individuals from training videos and generalizes to identify them in a group. [7]JAABA [5] was used to learn complex behaviors such as following and chasing (for details on JAABA [5] see Ctrax - note 6). [8]Social development in groups of mice [17]. [9]In their standard configuration (1024 x 768 pixel resolution), they fit a small rectangle (50 x 100 pixels) around each mouse to extract features needed for individual identification. With these specifications, each mouse body formed an ellipse with a long axis ~30-85 pixels, and the small axis ~10-45 pixels.

Multitrack (Movie S20)

Availability and usability: open-source, simple to use, flexible[1]
Raw output: 2D trajectory, orientation
Requirements: Linux, additional libraries[2]
Maximum individuals: 1000[(note 3)]
Shape requirements: flexible[4]
Identity method: not maintained[5]
Analysis tools: none[6]
Taxa studied: ants, bees[7]
Required resolution per individual: 3 pixels[8]

Notes: [1]Avaliable online at www.bio-tracking.org/category/software. Flexible to different setups. Can use conventional cameras. Requires constant background. System packed with companion programs for creating shape models and backgrounds for different experimental setups. [2]OpenCV and PCL (standard computer vision libraries), and Qt 4.0 (for creating graphical user interfaces). All three are cross platform and freely available. [3]Maximum tested ~1000 individuals, but can track an arbitrarily large number. [4]Individuals are assumed to be rigid (see note 1). Different types of body shapes can be tracked simultaneously by creating multiple models. [5]A proportion of crossings can be solved with movement models. [6]Only produces tracking data. [7]Spatial dynamics of ant [18] and bee (B. Hrolenok, unpublished) behavior. [8]Can be set as a threshold.

Multi-Worm Tracker [19] (Movie S2)

Availability and usability: open-source, simple to use, flexible[1]
Raw output: 2D trajectory, detailed pose (real-time)[2]
Requirements: Win, LabVIEW Vision (NI) run-time license[3]
Maximum individuals: 80[(note 4)]
Shape requirements: worm-like[5]
Identity method: not maintained[6]
Analysis tools: basic kinematics, basic behaviour[7]
Taxa studied: nematodes, fly larvae[8]
Required resolution per individual: 160 pixels

Notes: [1]Available online at http://sourceforge.net/projects/mwt/. Requires unchanging and uncluttered background. Can use conventional cameras. [2]Includes position of centroid, body size (number of pixels), vectors defining long (and orthogonal) axes of the shape, skeleton (11 point line along midline), and outline of body (compressed bitcode stored as a string). Summary file can store when a stimuli occurred, and 13 simple statistics (number of objects, mean speed, mean size, etc.). [3]Choreography runs on Win/Linux/Mac. [4]Up to ~500 individuals with lower frame rate (~10-15 fps). [5]Optimized for *C. elegans*. [6]Identities not maintained – trajectories lost when animals touch. [7]Choreography is an offline basic behavioral analysis package (direction, velocity). Plugins can compute organism- or condition-specific behaviors. [8]Determinants of behaviour in *C. elegans* [19], neural biology of *Drosophila* larvae [20, 21].

PhenoTracker (Movie S21)

Availability and usability: commercial, easy to use, flexible[1]
Raw output: 2D trajectories, basic pose[2]
Requirements: Win[3]
Maximum individuals: 50[(note 4)]
Shape requirements: flexible
Identity method: maintained (size/markings)
Analysis tools: basic kinematics, social behaviour[5]
Taxa studied: flies, ants, fish, rodents[6]
Required resolution per individual: 200 pixels[7]

Notes: [1]Developed by TSE Systems. Available online at http://www.tse-systems.com/products/behavior/video-tracking-software/phenotracker/index.htm. Free trial version available. Software only or with integrated hardware. A constant and uncluttered background not required. Shadows, bedding material and objects can be present in the arena. Valid for conventional cameras and laboratory setups, including multiple arenas. Online support avaliable. [2]Identity not maintained if individuals not marked. Basic pose icludes head, center of body, and tail. [3]Server version also available, requiring web browser. [4]Maximum tested, but in principle could do more. [5]Basic measurements per individual, such as body velocity, distance moved, turning rate, time spent in zone. Basic analysis for social interactions, including orientation towards or distance from other individuals. Can do statistical analyses between videos. [6]See all publications at http://www.tse-systems.com/cgi-bin/refsearchn.pl?type=pub. [7]Requires 4 pixels per individual for position only.

SOS-track [22] (Movie S17)

Availability and usability: open-source, simple to use, flexible[1]
Raw output: 2D trajectory, detailed pose (real-time)[2]
Requirements: Win/Linux/Mac, Matlab
Maximum individuals: 1[(note 3)]
Shape requirements: flexible[4]
Identity method: not maintained[5]
Analysis tools: basic kinematics, sensory biology[6]
Taxa studied: flatworm, larvae, fly, fish, rodent[7]
Required resolution per individual: variable[8]

Notes: [1]Available online at http://sourceforge.net/projects/sos-track/. Valid for conventional cameras and laboratory setups. Suited for different organisms and arenas. Can track several arenas simultaneously. Ability to add and track accurate information about the environment-organism interaction. Ability to easily correct errors (e.g., head-tail swaps). Requires constant uncluttered background. [2]Includes position of centroid, head, tail, and midpoint, and skeleton, curvature, and total area. Real-time data does not include pose information. [3]Main package can track a single individual. Extendable to multiple individuals (Movie S17), but loses identity when individuals are similar (code available from authors upon request). [4]Wide range of size and shaped individuals can be tracked, including elliptical, worm-shaped, and legged organisms. [5]see note 3. [6]Low-level motorsensory measurements (body posture, kinematic variables – angles, velocities, distances) and sensory information relevant to the individual (i.e., system can map relevant points along the individuals body to the sensory landscape, such as local orientation relative to the sensory gradient, or stimulus intensity at the front of the animal). Automated basic behavior classification available for worm-shaped animals: turns and head casts. [7]Environmental control of behavior in *Drosophila* larva [22, 23], other taxa (unpublished). [8]Can be set as a threshold, although pose tracking impossible at low resolutions.

ZebraZoom [24] (Movie S18)

Availability and usability: open-source, simple to use, specific[1]
Raw output: 2D trajectory, basic pose
Requirements: Linux, Matlab, OpenCV, C++[2]
Maximum individuals: 7[(note 3)]
Shape requirements: zebrafish-like[4]
Identity method: crosses solved[5]
Analysis tools: basic kinematics, basic behaviour[6]
Taxa studied: zebrafish[7]
Required resolution per individual: 350 pixels

Notes: [1]Free online at http://sourceforge.net/p/zebrazoom/wiki/Home/. Limited flexibility to other setups. High-speed camera (~330 Hz). Probably requires tuning of several parameters to adapt it to a new set-up. Not ideal for interactions involving close encounters between individuals. [2]Tested on Linux. [3]Validation for up to 7 individuals, possibly system can deal with more. [4]Optimized for zebrafish larvae, probably not generalizable to many other fish species. [5]Tracking algorithm not robust to crossings - switching of identification between two larvae estimated to occur every ~109 s (density of 7 individuals). Solves crossings, making on average one mistake per animal every 110 seconds. [6]For each behavior detected, extraction of output parameters per swim burst (i.e, number of oscillations, tail beat frequency, duration, orientation, distance travelled, mean speed) and parameters also calculated for population. Attached behavioral cluster package includes an automatic (trained) classifier of behaviors in three classes: slow forward swim, routine turn, and scape. [7]Genetic and environmental drivers of behavior in zebrafish [24].

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Table S2. Criteria for describing the automated image-based tracking systems in Table S1.

Category	Description
Analysis tools	Does the system automatically (or semi-automatically) output higher-dimensional indices of trajectory and pose data, such as individual kinematics (e.g., body velocity, distance to wall) or between individual behaviors (nearest neighbor, relative velocity). Is there behavioral phenotyping available? What packages or add-ons are available to assist with behavioral analysis?
Availability and usability	Where can the system be obtained? How easy is the system to adapt to different experimental setups and how easy is the system to use?
Shape requirements	What body shape is the tracking system optimized for?
Taxa studied	List of taxa that have already been tracked with the system, and corresponding publications.
Identity method	Is the identity of individuals maintained through occlusions, or are they solved using movement models (see Box 3)? If they are maintained, then is this through i) markers, ii) body size, or iii) other more specific morphological or behavioral traits that we group under the umbrella of fingerprinting. See Figure 1 (main text) for more information about identity maintenance.
Required resolution per individual	The approximate minimum size of individuals required for the system to perform in the way described in Table S1 (e.g. maximum number of individuals, ability to solve crossings, etc.). It is measured as the number of pixels that cover the area of an individual, averaged across a video in which the animals are as small (relative to arena size) as possible for the system.
Maximum individuals	Maximum number of individuals that can be tracked.
Raw output	Does the output of tracking include trajectories in 2D or 3D? Is any pose data collected, such as orientation or more detailed pose? Does the system run in real-time.
Requirements	What OS and any other software does the system require? Do you need any special imaging equipment, such as multiple cameras?