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# A microcomputer-based system for real-time analysis of animal movement

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**Abstract**

A microcomputer-based video system for tracking, recording, and analyzing the movement of animals in two dimensions on variegated background in real-time has been developed and validated, both mechanically and with moving animals. Hardware and software (donationware) costs are low. Specimens visualized as small as 3 pixels long may be tracked in an arena that is 240 × 320 pixels in size. The results of tracking a mechanical moving spot are compared with a theoretical circular path. Comparisons between frame-by-frame human observation and computer generated $X$–$Y$ coordinates are also presented. Applications of the tracking system include insect toxicology and pheromone bioassay, vertebrate locomotion studies, and basic research on taxes and kineses.

*Keywords:* Orientation; Movement pattern; Image subtraction; Motion analysis; Open-field behavior

## 1. Introduction

Analysis of animal movements in response to chemical, physical, and social stimuli is useful in basic and applied research. Studies ranging from pheromone bioassay and behavioral toxicology to searching behavior theory use motion analysis. However, precise recording and analysis of an animal's path is difficult, particularly if the rate of movement and change in direction are also of interest. Hader (1991) and Wratten (1994) edited volumes that covered video techniques as applied to ecology and behavior studies of nematodes, arthropods, molluscs and vertebrates. Bell (1991) discussed the temporal aspects and problems of path analysis in detail. There are many measures of movement, nearly all of which are more accurate if the sampling rate is high. Recognition of a specific response is most valuable if it can be identified as it occurs. An effective tracking system must follow movements on a photographically irregular background and provide real-time analysis and read-out. Additional desirable qualities of a tracking system are high resolution, low cost, easy availability, and applicability to many animal species.

Computer analysis of video images of moving animals was developed by Miller et al. (1982). Microcomputer-based systems developed by Godden and Graham (1983), Hoy et al. (1983), Dusenbery (1985), and Ye and Bell (1991) have utilized inexpensive and readily available hardware. Vigneault et al. (1990) described a system for measuring moving air bubbles in real-time which has been adapted subsequently to tracking moving insects. Although relatively inexpensive, these earlier microcomputer systems required special lighting conditions or high contrast between the subject and its background. Commercially available systems (e.g., Columbus Instruments, Motion Analysis, Leica) have become available, but remain quite expensive and are seldom reported in the scientific literature.

The purpose of this paper is to describe and validate a video-microcomputer tracking system that can follow an animal in two dimensions and satisfies the following criteria: (1) creates a data file of $X$,$Y$ coordinates, time, locomotory rate, and rotation rate of a subject moving on a variegated background, with observations every 0.2 s or faster; (2) provides readout of locomotory rate and turning rate in real-time at 1 s intervals; and (3) allows the user to

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define specimen size, observation interval, length of file, and to display a graph of the recorded path and statistical analysis of the data file.

## 2. Materials and methods

Lighting conditions dictate the type and cost of the video camera used with this system. The camera must be fixed for the image subtraction algorithm to function. Either live video or recordings may be analyzed.

The animal movement analyzer system has three components: a video camera, a microcomputer, and a software program. The critical components of the system are an Apple Macintosh Quadra 660AV and a 'donationware' program (combined cost of about $2300).

### *2.1. Hardware*

This system was developed using an Apple Quadra 660AV computer which has a built-in digital signal processor (AT&T 3210), 8 Mbytes of RAM, and a 500 Mbyte hard disk. Validation of the system was done with a Computar TV 8-mm lens and a Cohu model 4810 video camera capable of operating at low light intensity (0.015 lux) and producing an NTSC output video signal with 512 horizontal by 384 vertical lines of resolution. Both overhead flurescent lighting and light from red darkroom incandescent bulbs were used during testing. Certain parts of the validation utilized a Sony model SLV-373UC VHS video cassette tape recorder and a Panasonic model WJ-810 time-date generator.

### *2.2. Software*

The program was developed jointly with Electronic Learning Systems (Gainesville, FL) using Symantec C + +, version 7, System 7, QuickTime, version 1.6.1, and 1 Mbyte of available dynamic memory. (See Hoy (1994) for details of the system.) The copyrighted software (Hoy et al., 1993) is available as donationware through the Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611-0620. The basis of the central algorithm is image subtraction of a reference video frame from subsequent frames as the test subject moves about the camera's field of view. The computer monitor simultaneously displays three windows, one with the live picture from the camera, one with a trace representing the $X,Y$ coordinates of the moving subject as they are stored, and one with digital readout of speed and turning rate. While a subject is being tracked, the live window shows the subject with a flashing tracer spot superimposed upon it at each recorded observation. The location of the subject is recorded in a computer file at user specified intervals up to 7.5 frames per second (FPS). Each location is recorded as an $X,Y$ coordinate along with the frame number, the elapsed time, and a running average of the subject's speed and rotation rate. Speed is calculated by dividing the Euclidean distance by elapsed time. Rotation rate is calculated by dividing angular change by elapsed time.

The video signal is digitized in a $240 \times 320$ pixel format (bitmap). Calibration of the system simply requires knowing the width of the visualized arena; camera and lens specifications are relatively unimportant.

Recorded files may be replayed. During the replay, the path is displayed as a graph; the means and standard deviations of the speed and rotation rates are also calculated and displayed. The file is in ASCII text, and can be imported by a spread-sheet program for analysis beyond the real-time analysis or the analysis provided in the replay utility.

An additional utility program accumulates deviations from the initial values for each pixel of the reference frame over a user-specified time. By recording changes in the *absence* of a moving experimental subject, electronic or photographic variation can be identified and eliminated by adjusting the system parameters or lighting.

Prior to data acquisition the user specifies parameters by which the computer can identify the moving subject. First the user chooses between a dark subject on a lighter background or the inverse. Size and degree of contrast with the background (gray threshold) must be specified. The gray threshold is the minimum difference that will be recognized as a change of status between the value for a given pixel in the reference frame and the frame being analyzed. The sampling rate (up to 7.5 FPS) and length of observation are then set, followed by a file name and notation. The parameters of the recording are automatically included in each file header. The contents of the file may be exported and analyzed for information other than the calculated speed and rotation rate.

Subsequent to development and validation of the first version of the program, we have a translation of the code into Power Macintosh native code, using Symantec C + + and a Power Macintosh 7100AV.

### *2.3. Test subjects*

Male German cockroaches, *Blattella germanica* (L.), were used in validation experiments, except for the mechanical test and simple tests of a light specimen on a darker background (a predacious mite), and a test of a small elongate specimen (a flea larva).

### *2.4. Validation*

For a mechanistic test of the system we used a turntable with a theoretical speed of 33.33 r.p.m. and a 15-mm spot 12.7 cm from the center of the turntable. The position of the spot was recorded at 6.0 FPS (6/s) for 60 s. The 25.4-cm diameter of the circle created by the moving spot was visualized by the camera as having a diameter of 124.5 pixels (4.90 pixels/cm).

The relationship between the actual path of a living test subject and the digitally recorded $X,Y$ series was the primary basis of system validation. Validation was done at 7.5 FPS.

Electronic and analytic variation was estimated by recording a dead cockroach for 1 min in 3 square arenas (22.5, 45, and 90 cm) and 5 gray thresholds (20, 24, 32, 40, 48 shades). Recorded movement (apparent movement of the dead specimen) represented changes in the image or analysis of the image through time, and is a measure of the accuracy of the results. Tracking ability, as influenced by contrast settings (gray threshold) and the visualized size of the specimen, was examined by finding the limits of error-free tracking of a motionless specimen.

Frame-by-frame comparison of the positions of the subject on a grid, as judged by a human observer that was unfamiliar with the computer record versus the $X,Y$ coordinates recorded by the computer, established the accuracy of the program. Thirty consecutive segments from a recording of a moving cockroach were compared. The observer estimated the center of the specimen and its location to the nearest 5 mm on a $25 \times 25$ grid of 25-mm squares. The camera position resulted in 1 mm of the arena equal to 0.53 pixels, the 15-mm specimen visualized as about 8 pixels long, and the position recorded $\pm 1$ mm. The human and computer observations were synchronized by comparing time-stamped frames of a video tape with the time stamp in the computer recorded file. The absolute difference in pixels between the two locations was then calculated.

## 3. Results and discussion

The 60-s record of the moving spot on the turntable resulted in 33.65 observed revolutions in 60.07 s. The theoretical speed of the spot was 217.1 pixels/s with an expected 360 observations. The computer system recorded a speed of 216.7 (SD 1.64) pixels/s with 348 observations. The theoretical rate of rotation was 200.00°/s. The computer system recorded rotation of 202.00° (SD 3.48), with 348 observations. The difference between 360 expected observations and the 348 recorded probably was the result of short delays in processing the video image. The low standard deviations in both speed and rotation are consistent with the uniform speed and rotation of the turntable.

Initial testing of the system using living animals determined that cockroaches 15 mm in length in a 1 $m^2$ arena were visualized on the live screen as 3 or 4 pixels long in a $240 \times 240$ pixel arena. During recording of a path, the superimposed tracer indicated where the specimen was as its location was recorded. Replay of the file and inspection of the graphed path confirmed that the entire path had been recorded. Subsequent tests were done with the vertical camera positioned so that 90, 45, or 22.5 $cm^2$ arenas were visualized as $240 \times 240$ pixels. Specimens were visualized at 4, 8, and 16 pixels, respectively.

Table 1
Erroneous records of movement of a motionless cockroach, according to arena size and gray threshold setting

| Arena size (cm) | Gray threshold | Reported speed (pixels/s) | Moves ($n$) | Move size (pixels) | Move/specimen size |
|---|---|---|---|---|---|
| 22.5 | 24 | 0.83 | 23 | 1 | 1:16 |
| | 32 | 0 | 0 | 0 | – |
| | 40 | 0 | 0 | 0 | – |
| | 48 | 0.10 | 3 | 1 | – |
| 45 | 24 | 0.52 | 14 | 1 | 1:8 |
| | 32 | 0 | 0 | 0 | – |
| | 40 | 0.66 | 20 | 1 | 1:8 |
| | 48 | 0.77 | 21 | 1 | 1:8 |
| 90 | 24 | 0.03 | 1 | 1 | 1:4 |
| | 32 | 0 | 0 | 0 | – |
| | 40 | 0 | 0 | 0 | – |
| | 48 | 0 | 0 | 0 | – |

Variation in the recorded location of a motionless (dead) specimen was limited to 1 pixel under any condition that the program could 'lock on' and record locations. At intermediate gray threshold settings (24–40 shades) there was limited variation. The results in terms of erroneous speeds (jitter) reported for a dead specimen at gray thresholds which defined the range that avoided jitter are given in Table 1. At a threshold of 20, the program could not locate the specimen at any distance. At a threshold of 32, recording was without variation, regardless of the distance. Furthermore, at the extreme gray threshold (48), jitter was absent when the specimen image was smallest. The ratio of the size of the jitter relative to the length of the 15 mm specimen was 1:16 in the 22.5-cm arena, 1:8 in the 45-cm arena, and 1:4 in the 90-cm arena. In general, jitter can be avoided at a threshold of 32, and is of small magnitude when it does occur.

Establishing the accuracy of the program when analyzing the movement of a living specimen by comparing the recorded coordinates with frame-by-frame estimates of location by a human observer opens the question of accuracy of the observer. This analysis initially assumes that the human observer is totally accurate, a questionable but conservative assumption. The path of the specimen, based on computer and human observations, is shown in Fig. 1. The looping path is predominantly a long turn at varying speeds with meandering midway and near the end.

The difference between the human and computer observations ranged from 0–6.5 pixels on the $X$ axis and from 0.5–8.5 pixels on the $Y$ axis (Fig. 2). $Y$ axis differences were positive in all but 3 cases. Inspection of Fig. 2 suggests a $Y$ axis bias of approximately 4 pixels, or about one half the body length of the test specimen. The computer's determination of center of mass or human observa-

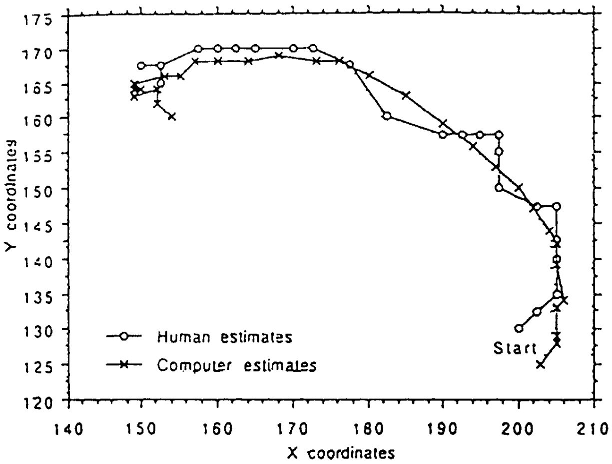


Fig. 1. Path of cockroach through first 30 observations, as recorded by a computer, and as reconstructed by a human viewing a video tape recording frame-by-frame. $X$ and $Y$ coordinates are in pixels.

tion may account for the bias. There is no apparent bias on the $X$ axis.

Once the basic method of recording had been validated, the tracking of the inverse of a dark specimen on a light background was tested, as was the tracking of an elongate specimen. Validation of the part of the program that allows tracking a light specimen on a darker background utilized a phytoseiid mite on a slightly darker bean plant leaf. As in the initial test, the specimen was located and successfully tracked, as confirmed by the tracer during the run and by the replay option of the program. As an alternative to the compact shapes of the cockroaches and the mite, a flea larva with a length to width ratio of about 8 : 1 was also successfully tracked. Single-frame analyses of the mite and flea larva recordings was not done.

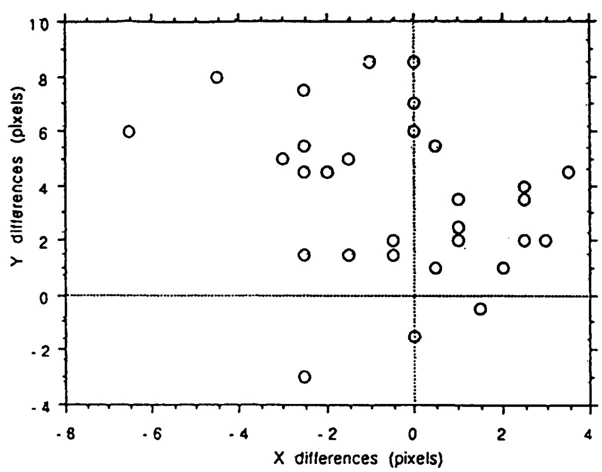


Fig. 2. Scattergram of differences between computer and human observation of a moving cockroach. The differences were calculated by subtracting the computer estimates from the human estimates of both the $X$ and $Y$ coordinates. Differences on the $Y$ axis were biased in a negative direction, i.e., computer estimates were lower than those of the human observer.

Sources of error in the recorded path occur at each step in the process. However, optical errors such as lens distortion are beyond the scope of this report. Electronic noise can result in changes in the value of a single pixel, but those errors are minimized by the algorithm that identifies the specimen. Errors resulting from poor definition of the specimen are negligible within the middle range of gray thresholds, as shown in Table 1. Although the human observer was assumed to be totally accurate, some of the differences between the human and computer observations may have come from errors in perception or the estimation to the nearest one-fifth of the grid units. One-fifth of a grid unit was equal to $\pm 2.5$ pixels. Also, estimation of the center of the specimen could have been off by $\pm 1$ pixel. Synchronization of the video tape which had time stamps at 0.01 s and the computer file which was time-stamped at less than 0.02 s could have resulted in errors of less than 0.4 pixel.

The lines on the graph paper substrate for the cockroach tests and leaf veins in the substrate for the mite test provided moderately variegated backgrounds. The brown cockroach contrasted greatly with the light-green graph paper, but the light-colored mite contrasted little with the medium green of the leaf substrate. In both cases, the specimen was readily tracked.

Finally, the locomotion and rotation rates are reported as running averages of the past 5 observations, therefore there may be lag and/or surge effects within about two-thirds of a second when recording at 7.5 FPS (5 observations).

The validation described above has been limited to general characteristics of the system. Lighting, camera characteristics, background, and the test subject's traits all influence the ability of the system to recognize the subject and record movement accurately. The tracer feature offers real-time confirmation of the integrity of the record. In general, diffuse light is helpful and shadows cause problems. If the subject is compact, sharp focus is helpful, but if appendages are large, poor focus can keep the tracer centered. Where the performance of individual specimens is critical, repeated analyses of videotape record would seem advisable.

## 4. Applications

This system appears to be suitable for recording and analyzing movement in two dimensions where the background is variegated but unchanging. It is currently in use for cockroach behavior studies and could be used for insect toxicological and pheromone studies, and vertebrate behavior studies. The program is being tested in another laboratory for tracking 3-dimensional movement by linking two systems with cameras set on perpendicular axes. Demonstration copies of the software are available on request.

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