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A microcomputer-based activity meter for multiple animals

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Abstract

We describe and validate a computer-video system that records and displays in real-time the activity of multiple specimens in a user-defined space, at user-defined intervals. The computer program uses image subtraction algorithms to record changes in video images, and stores observations in column format or as a series of 2-D matrices. The program was tested under various lighting conditions, backgrounds, specimen size and specimen speed. An AV model of Macintosh computer with video input from a camera or video cassette recorder was used to record and analyze the mechanical movement of spots on a turntable and the locomotor activity of an ant colony. The limitations and potential applications of the program are discussed. © 1997 Elsevier Science B.V.

Keywords: Video image analysis; Locomotor activity; Open field behavior; Computer motion analysis

1. Introduction

Quantification of animal activity in space and time is needed for such studies as orientation, circadian rhythms, locomotor effects of toxicants, and monitoring bioassay systems and animal colonies. Abramson (1994), Hader (1991), and Wratten (1994) give overviews of activity quantification methods. Activity wheels, actographs, and shuttle boxes are widely used, but those devices generally ignore space as a factor (e.g. Lipton and Sutherland, 1970; Leppla et al., 1989). Devices utilising electronic or infra-red beam grids (Kelley, 1993) address the space factor, but have poor resolution, have small arena size, and are very expensive.

A microcomputer coupled with a video camera facilitates measurement of activity, with high resolution in space and time. Some systems provide analysis of multiple specimens (Dusenbery, 1985). Fleury et al. (1991); Allemand et al. (1994) describe a system that provides real-time analysis, and robotic camera control, thus enabling sequential observation of individual specimens. However, there is a need for a simple and inexpensive system that records the activity of many experimental animals simultaneously, according to areas of activity, without regard to the paths of individuals. The Apple Macintosh and Apple QuickTime as a system base avoids the cost of a digitizing board, thereby limiting the system cost to approximately $3000 (US). We are not aware of a comparable Windows based system, nor do we at this time anticipate a Windows version of our software.

The purpose of this paper is to describe and validate a Macintosh-based computer-video system that simultaneously records and displays the activity of multiple specimens moving in two dimensions on a heterogeneous background, at user-defined intervals. The copyrighted software used is entitled 'Multiple Animal Movement Analyzer' (Hoy et al., 1995). The software design criteria were that the user could define sampling intervals, sample every second, adjust spatial resolution,

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and recognize movement occurring within as little as 0.15 s. Furthermore, the user could choose to record the distribution of activity, summed for an entire series of observations, or to record activity over specific time intervals.

The following questions will be answered: What factors in the experimental set-up are important for effective use? What parameters of the recording program are important under selected experimental conditions? What limitations in the system as a whole are apparent?

2. Materials and methods

2.1. Hardware

An Apple Macintosh 660AV computer and a Cohu model 4810 video camera were used to validate the Multiple Animal Movement Analyzer. For repeated analysis of specific movements at various tracking parameters, a Panasonic (model AG-6750A) video cassette tape recorder provided a video input. Subsequently, additional validation tests were done using a Power Macintosh 8100. All experiments were done under incidental overhead florescent lighting. The 64 × 48 data storage format (5 × 5 pixel cells) makes the resolution of the camera and recording equipment relatively unimportant so long as the resolution is not less than 320 × 240 lines. (This data format was chosen to allow real-time analysis and display).

2.2. Software

The program, which runs on any AV model of Macintosh computer, was developed jointly with ELS, (Gainesville, FL), using Symantec C + + and QuickTime version 1.6.1, subject to our specifications and validation. The program is copyrighted, and is available as shareware through the Department of Entomology and Nematology, University of Florida, Gainesville, FL. Validation was done on a Macintosh 660AV with NTSC video input, but PAL and SECAM input can be used. An image subtraction algorithm compares a reference video frame with a subsequent frame and stores the differences according to user-defined subdivisions of the video picture. The frame may be subdivided into halves, quadrants, or cells progressively smaller down to a 64 × 48 grid that has 3072 cells, each 5 × 5 pixels in size. Also under the user's control is a gray threshold filter for contrast between the experimental subjects and their background. The user can also adjust the intervals between frame comparison, and observation intervals between comparisons. A scaled value for the number of pixels within each cell (picture subdivision) that change value more than the user-defined threshold are recorded at each observation.

The user may choose either a 'distribution meter' that accumulates all changes through an extended observation period or an 'activity meter' that records motion over specified periods of time. Each activity meter observation is a 'snapshot' image of motion (a reference frame paired with a comparison frame).

2.3. Validation

Experimental set-ups impose constraints such as lighting, variegated substrate, specimen size, and specimen speed on recording of animal activity. Validation of mechanical movement was done under incidental lighting, on a uniform dark gray background, with a 5 pixel diameter white spot moving at 10 ± 0.5 pixels/s. The moving spot was located 12.5 cm from the center of a turntable that revolved at 1 rpm. The speed and path of the spot was confirmed using a computer program for tracking single specimens (Hoy et al., 1996). The multiple animal tracking capability described herein records changes in the video picture in a matrix that represents the whole picture rather than the path of a moving single object. Subsequent to the initial test with a single spot, a second and third spot were added to the turntable. Recording a spot moving at about two times the diameter of the spot is equivalent to a 1.5 cm cockroach walking 3 cm/s, a moderate pace for a cockroach.

Biological specimens rather than mechanical movement were also analyzed. Ant colony activity was recorded to demonstrate the use of the program to clarify changing foraging patterns during a time series. Individual ants (*Solenopsis invicta* Buren) were visualized as approximately 2 pixels long in a 320 × 240 pixel bitmap of the video picture. During six series of observations (at 2 min intervals over 10 min) as few as 100 and as many as 500 ants were active. The entire area of the test arena was within the camera's field of view. The ant colony was under incident florescent light on a laboratory bench.

3. Results and discussion

Video-based measurement of activity generally requires adequate and relatively uniform lighting. Although image subtraction can compensate for a photographically heterogenous background, some degree of contrast between the moving subject and the background is required. Our validation was done under casual lighting, but with a sharply defined 'subject' on a high contrast background. The recording parameters that were tested and their relative importance are described in the following four sections:

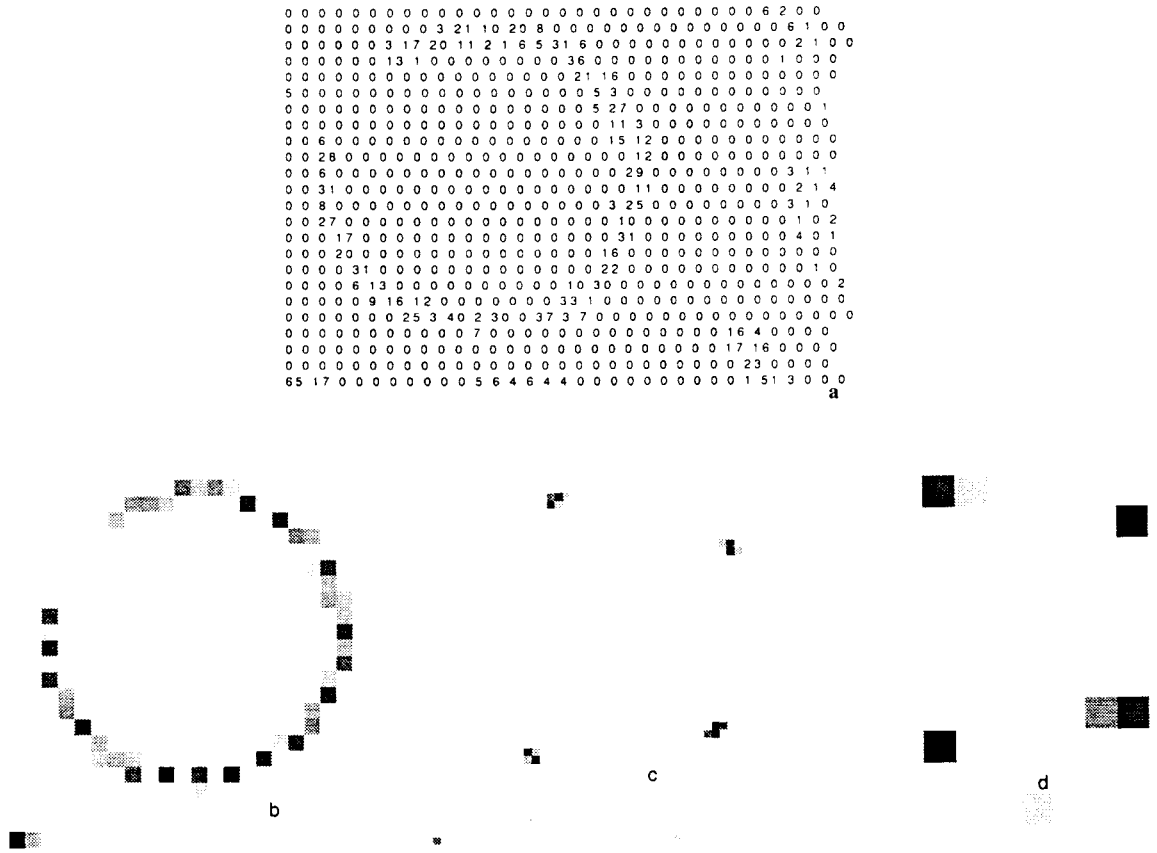

Fig. 1. Illustrations of data recorded for a spot moving on an arc at 6° per s. (a) A numerical data file, in matrix format, that shows the summarized locations and amounts of pixel change during 26 observations at 2 s sampling intervals of a 5 mm spot moving 10 pixels/s, with the arena subdivided into 768 cells. (b) A 'picture file' of the locations and degree of pixel change during the 26 observations in the matrix with 10 × 10 pixel cells. (c) An example with 5 × 5 pixel cells. (d) An example with 20 × 20 pixel cells. The picture files are useful in finding foci of activity, but in fact they are graphic representations of numerical data.

3.1. Gray threshold (contrast)

Our software works with computers that record contrast with a standard 256 shades of gray. Preliminary tests showed that a moving white spot was distinguished from the background over a wide range of threshold values, no clear difference between 60 and 80 shades of gray. Below 60, non-moving artifacts were sometimes recorded as movement and, well above 80, there was danger of not recording true movement in poor light. With very good light, records can be made at a threshold of 120. Therefore, all subsequent tests were done with 70 as the threshold value.

3.2. Data matrix cell size

The moving spot analyzed by our system was roughly representative of an animal moving in an arc at a uniform speed of 2 body lengths/s. The results of the accumulated record of 26 observations at 2 s intervals of a spot moving at 10 pixels/s (1 rpm) are shown in Fig. 1. Both the data matrix (Fig. 1a) and the graphic record (Fig. 1b) are as stored when the analysis is done with the video picture analyzed using a 32 × 24 grid. Each value represents a 10 × 10 pixel portion of the picture. The record of movement at the lower right and upper left margins of the matrix are respectively the changing time stamp and a flicker at the margin of the picture. Fig. 1c and (d) illustrate the results of recording a 5 mm spot four times (at 12 s intervals) using cell sizes of 5 × 5 pixels and 20 × 20 pixels respectively. In both cases the comparison interval was 0.5 s. Cell size can be small if the subjects are small or slow moving.

3.3. Comparison interval

The comparison interval, the elapsed time between the two video pictures used in the image subtraction process, may be as short as 0.15 s. The image subtraction process records where the specimen was at the beginning and end of the comparison. Fig. 2(a,b) illustrate the effect of lengthening the comparison interval from 0.5–3.0 s. When the speed of the subject is such that it moves more than two 'body lengths' during the

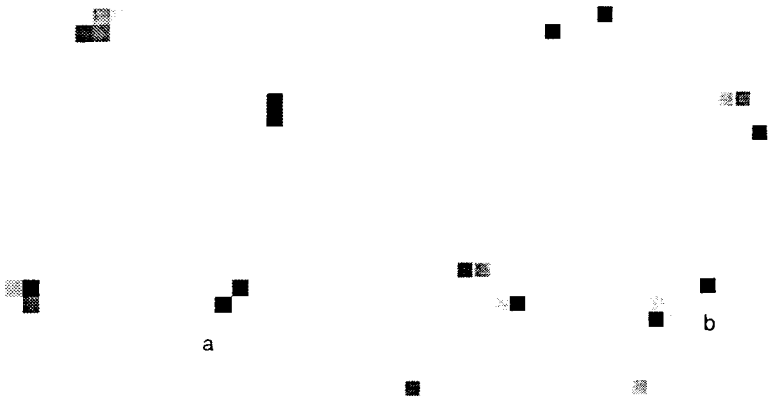

Fig. 2. Illustration of the effect of the comparison interval between paired frames of four observations at 12 s sampling intervals. (a) Comparison interval, 0.5 s. (b) Comparison interval, 3.0 s.

comparison interval, a gap occurs in the record. Note the gaps in the four observations (at 12 s sampling intervals) recorded in Fig. 2b, whereas the 0.5 s interval illustrated in Fig. 2a captures all movement and records it in contiguous cells. Thus, a 5 pixel subject moving at 10 pixels/s is recorded as a single elongate trace, assuming that the cell size is greater than the length of the specimen. An appropriate sampling interval depends on specimen size and speed, with consideration for cell size. For best results, the comparison interval should be long enough to detect motion, but short enough so that subjects do not move more than two body lengths during the interval. Within these constraints our system records activity where and when it occurs. Comparison interval is the parameter that is most important because it requires consideration of the characteristics of the experimental subjects and the experimental set-up.

3.4. Intervals between comparisons

Samples (observations) may be taken at intervals of 1 s or greater. Fig. 3 shows the cummulative results of sampling a spot moving at 10 pixels/s, four times at 1.5, 3.0 and 6.0 s intervals. Sampling interval choice greatly affects the size of data files, but is relatively unimportant unless the experimenter needs to record rapid fluctuations in subject activity. The user may also choose a series of observations with long pauses between series.

3.5. Multiple moving objects

The effects of the various recording parameters described above are best demonstrated by a single spot. We extrapolated the validation method to observation of three spots. Eight observations at 4 s intervals of three spots moving in concentric circles, as illustrated in Fig. 4, show the record of multiple objects that may be achieved. Examination of the data matrix upon which the graph is based revealed that the outermost spots had combined cell values ranging from 25 to 32, the middle spots had values from 22 to 31, and the innermost spots had values from 19 to 27. The differences among positions no doubt reflected differences in speed due to the distances from the center of the turntable. Furthermore, the values for a given spot were greatest at the ends of the rotational cycle, which suggests that contrast was least along the lower right portion of the arc, with a difference of about 25% between the high and low value for a given position. These results suggest that activity is recorded accurately with respect to location and time of occurrence, but that the degree of activity within a cell is not precisely measured.

3.6. Ant colony activity

The rapid change in formation of a red imported fire ant foraging trail in a laboratory colony over a 10 min period is shown in Fig. 5. This example of the use of our system also shows the results of six series of 15 observations at 2 min intervals. The data were recorded at the maximum resolution of the system, i.e. a grid of 64×48 cells. (See the figure caption for comparison interval and sampling interval details). The data were then imported into DeltaGraph Pro (version 3.5) and the figure was constructed using the XYZ contour fill option.

The ant colony used in this test was in a plastic tray that provided relatively good contrast between the ants and the substrate. Light tables that provide transmitted light are a reasonable laboratory alternative. Field use of this system on ants of this size would be limited to an area of about 0.5 m^2, and where lighting did not change during the interval between paired reference and observation frames.

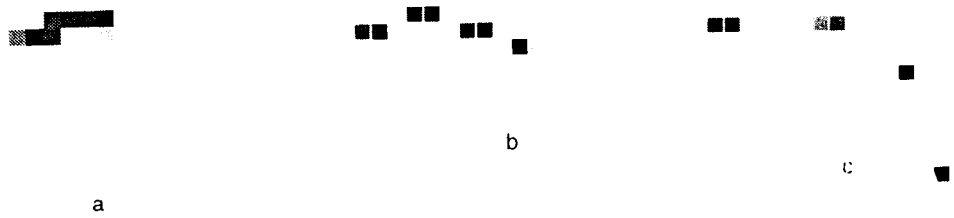

Fig. 3. Illustration of the effect of increased sampling interval. (a) Sampling interval, 1.5 s. (b) Sampling interval, 3.0 s. (c) Sampling interval, 6.0 s.

The efficiency of capturing movement is difficult to determine. This system records the location and time of activity. But, without calibration against a standard, the values stored in a given cell are subjective.

3.7. Applications and limitations

We believe that this system offers a method of continuously recording animal activity on a heterogeneous background with high resolution in time and space at a relatively low cost. The components of the system, an AV model Macintosh computer, video camera, and the software herein described are available at approximate costs of $2000, $200 and $450, respectively. The system weighs approximately 25 kg, including the computer monitor, requires AC power, and occupies less than 0.5 m^2 desk space. The use of an image subtraction algorithm permits use of the system where the background is varied. With uniform lighting and a stable camera, field use would be possible, but activity recorded on video tape for analysis in the laboratory might be a better alternative. The study of the influence of toxicants, food sources, attractants and repellents on the behavior of an insect colony was the original purpose for the system's development. Applications could range from investigation of circadian rhythms to open field or maze studies.

The primary limitation of the system is that uniform lighting is required to avoid bias when low gray thresholds are used. Also, rapidly moving subjects will result in under reporting of activity. Finally, the system does not record activity of individual animals, but rather group activity within specified two dimensional areas.

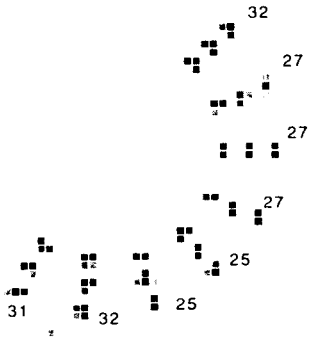

Fig. 4. The cummulative record of three spots observed eight times at 4 s intervals. The combined recorded values for each of the outermost spot have been added to show the variation in value through the arc of observations.

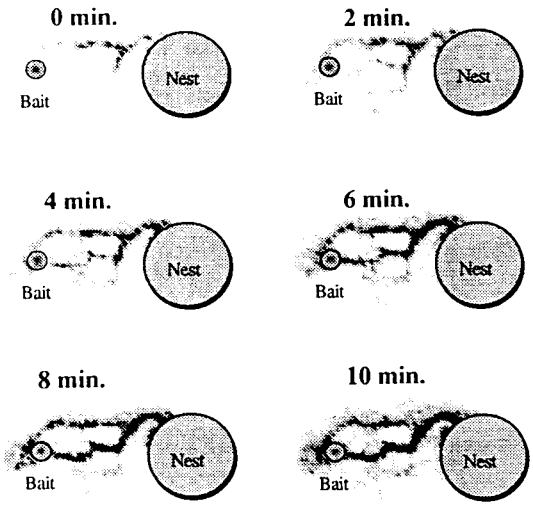

Fig. 5. Formation of a fire ant trail, at 2 min intervals. Each part of the figure is the sum of 15 activity samples, taken at 5 s intervals with a frame comparison interval of 0.4 s. The observation field was subdivided into 3072 cells. This figure was constructed using XYZ contour fill option on DeltaGraph Pro (version 3.5).

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