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Video-Based Rodent Activity Measurement Using Near-Infrared Illumination

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Abstract - *This paper describes a non-invasive video tracking system for measurement of rodent behavioral activity under near-infrared (NIR) illumination. This novel method allows tracking of motion in the dark, when rodents are generally most active, and also under visible light. Results of the system were validated through comparison with simultaneously recorded data using a widely-used NIR crossbeam-based tracking system.*

Keywords - *video-based rodent measurement, near-infrared illumination, non-invasive tracking system, image processing*

I. INTRODUCTION

Observation of rodent behavior is a useful and prevalent method for elucidating the effects on rodents of various experimentally applied conditions, including drugs of addiction, novel disease treatments, and genetic mutations. During observation, the identification of many different behaviors may be desired, such as walking, grooming, rearing, etc [1]. Therefore, video tracking and analysis is advantageous due to its ability to show very specific behaviors and to also allow the opportunity to record the video and analyze it in the future for as-yet undefined behaviors. Examples of systems developed for video monitoring of rodents are the commercial products Activity Monitor 5 (MED Associates, Inc.), EthoVision ® (Noldus Information Technology) [2], and the PhenoScan series (CleverSys, Inc.), and systems developed in the academic sector [3][4]. Other notable examples in the literature, not specifically for rodent tracking, are [5], which relies on shape differences for identifying object differences, and [6], which demonstrates a method for tracking multiple interacting objects.

Because rodents tend to be most active at night [7][8] the difficulty of video tracking in both light and dark conditions increases the complexity of capturing a large and possibly useful volume of data. Recent research has shown the similarity of rodent behavior in the dark and under 940nm infrared illumination [9], therefore the use of such illumination would have little influence on the rodent's dark-cycle behavior, and is therefore non-invasive. However, most of these systems are configured for dark environment tracking. Near-infrared (NIR) illumination (light with wavelengths from 700nm to 1100nm) presents difficulties for tracking because it can distort color intensity information (due to objects having different NIR reflectances than under visible light). Also, when the focal length of an optical lens is set for NIR use, it is not optimal for wavelengths of light in the 350-600nm range. The former often results in objects having different color characteristics than would normally be seen, and the latter produces a tendency toward blurred edges when the light source is switched without re-focusing the lens. Although [2] has recently been updated for use with NIR, it works best with constant round-the-clock NIR illumination in addition to visible light, which wastes energy and can produce unwanted heat in the rodent housing system. Here, we have further developed a practical and inexpensive method for video tracking in the dark, based on [9], using a high-quality digital video camera without an infrared filter (which is present in most cameras to improve visible pictures) and NIR illumination. Therefore, we developed two algorithms to overcome this problem [9]; with them we can find and confirm the rodent location easily.

The topic of this paper is the validation of video based rodent activity measurement in an open field chamber, illuminated by either NIR or visible light. Open field activity monitoring involves placement of the rodent into a chamber devoid of any contents and recording motion and other activities of interest (i. e. wall-hugging which is referred to as thigmotaxic behavior, a potential indicator of anxiety). The validity of this measurement technique is demonstrated by correlation with results from beam breaks in a NIR LED array. This system contributes to both the fields of video-based measurement and behavioral research by providing a novel method of activity measurement in the dark that improves on the work of [9] in that a rodent of low contrast to the background can also be tracked, as well as rodents under visible light. The results of this computer vision technique are then correlated with those of an NIR crossbeam tracking system (MED Associates, Inc. Activity Monitor 5).

II. PROPOSED APPROACH

A. Tracking system architecture

As in [9], a CCD camera system was interfaced via IEEE-1394 to a computer, with an adjustable array of visible and infrared LEDs fixed above the rodent chamber. NIR LEDs for recording beam-crossing (used for validating the results) were affixed to the lower perimeter of the system. A system diagram is shown in Fig. 1. This array can be switched between white light and NIR illumination for the light and dark monitoring, respectively.

To test this system, trials were run using 6 experimentally naïve Sprague-Dawley rats (Harlan International), each approximately 60-90 days old and housed in a reversed 12-

hour light-dark cycle (their low-illumination time was set during the day, hence "reversed"). Trials were run while the rodents were usually in their "dark cycle". Each trial consisted of 30 minutes spent in a standard open field activity-monitoring chamber (MED Associates, Georgia VT USA). Overhead illumination conditions consisted of visible light, 880nm NIR, 940 NIR, and "no light" (consisting of an extremely low level of 940nm NIR). The activity was evaluated quantitatively for distance traveled, average velocity, and time spent resting for both video tracking and the NIR crossbeam system.

B. Tracking Algorithms

The combination of algorithms used here relies on acombination of motion detection via image subtraction and color thresholding to confirm rodent location. Color was useful as a parameter because even though small differences in image color weren't easily observable in the NIR range, when analyzed on a pixel-by-pixel basis they provided more sensitive indicators of image change than an actual monochrome image. A time-shifted image subtraction algorithm was used to subtract the previously captured frame; this provided the ability to ignore changes in the chamber that had occurred since the beginning of the experimental trial, e. g. the addition of rodent feces.

Location Algorithm. The location algorithm (Fig. 2) was adapted from [9]. In this method, a region was first checked for motion. The size of the region was determined for the input requirements of Activity Monitor, for which the sampling distance of the number of rows and columns is based on the chamber size that is being used. If motion was detected, a color vector was computed as shown in Eq. 1:

$$P_c(r_i, g_i, b_i) = \sum_{(x,y)=R_i} \frac{P_c(r(x,y), g(x,y), b(x,y))}{N_i} \quad (1)$$

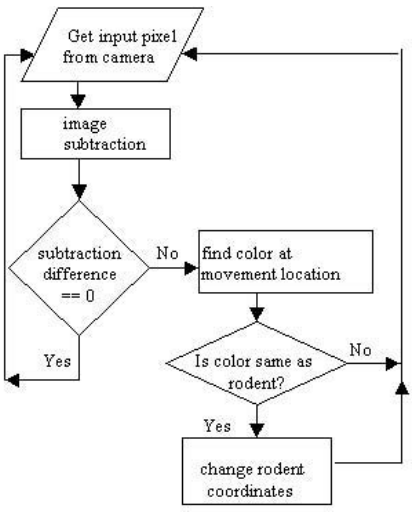

Fig. 2. Motion-based algorithm for rodent location

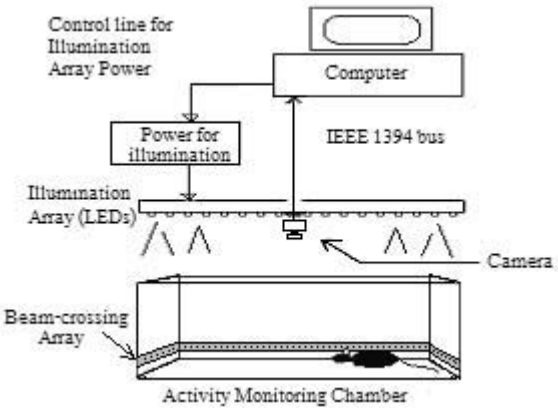

Fig. 1. Activity Monitoring System for Rodents

where the color pixel P_c has intensities of red = r_i , green = g_i and blue = b_i at location (x,y) in region R_i . This color vector was compared to the expected values using a goodness of fit ratio. Goodness of fit was determined as in [10] by calculating the ratio of each pixel color to its expected value, as in Eq. 2:

$$\psi_i = \frac{\max(P_c(\frac{r_i}{\overline{ri}}, \frac{g_i}{\overline{gi}}, \frac{b_i}{\overline{bi}}))}{\min(P_c(\frac{r_i}{\overline{ri}}, \frac{g_i}{\overline{gi}}, \frac{b_i}{\overline{bi}}))} \quad (2)$$

Where ψ_i is the goodness of fit parameter, r_i , g_i , and b_i are the measured color intensities and $\overline{r_i}$, $\overline{g_i}$, and $\overline{b_i}$ are the expected color intensities. If the goodness of fit was within acceptable limits, the location was marked. The new point was calculated using Eq. 3.

$$\begin{aligned} x_i &= x_{i-1} + (x_i - x_{i-1}) * 0.25 \\ y_i &= y_{i-1} + (y_i - y_{i-1}) * 0.25 \end{aligned} \quad (3)$$

This reduced "jumping" and smoothed the tracking of the rodent.

Confirmation algorithm. The change in color intensity of the target was used to confirm the location of the rodent. The pixel color of the target location was examined; the target location was determined to have "moved" only if one of the color intensity values (red, green, or blue) of the location had changed by more than 25 levels (out of 256 possible pixel intensity levels). This allowed us to find a rodent that was a similar color to the background, and tracked more smoothly. The algorithm speed is greatly increased with little or no loss in accuracy when a subsampling of just every 5th row is checked. If the pixel was below the threshold, a count was started to determine the length of the line. The minimum limit was set to the minimum visible area of the rodent during

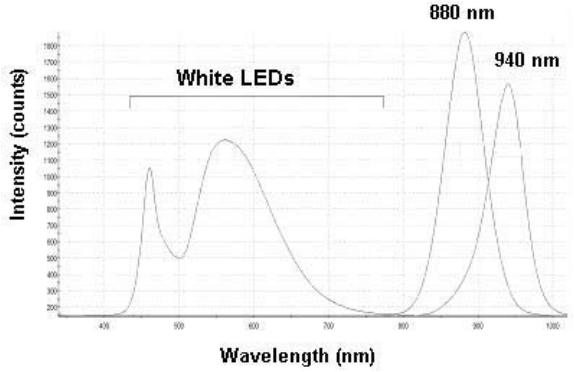

Fig. 3. Maximum spectral output of LED arrays at test height (~50 cm). The x axis represents light wavelength (350-1025 nm) ,and the y axis is a relative intensity measurement.

rearing, the maximum length to 105% of the rodent's length from nose to rump when stretched out. A marker was also drawn to indicate the location sent to the tracking program. Establishing minimum limits reduced noise, and maximum limits reduced the chance of the chamber sides being erroneously marked.

III. RESULTS

Before the trials, we measured the wavelength statistics of the NIR illumination used. The emitted wavelengths of the illumination LEDs were measured using a photospectrometer (Ocean Optics model USB2000, Dunedin, Florida). The spectral outputs of these are shown in Fig. 3.

The location of the rodent was determined using two methods: 1) Activity Monitor, and 2) our video system. The body of the rodent was located and transmitted to Activity Monitor by using the output of horizontal NIR crossbeams. Fig. 4 shows the superimposed output of both tracking systems for a representative trial. Since we were using rats more than two inches wide, limiting the search area to one inch from the walls greatly reduced rodent location error for our system, as the middle of the rodent was unlikely to be located less than one inch from the wall (the validity of this assumption is evident from Fig. 5). The mean distance reported by the two programs was not significantly different (paired samples t-test, t = .089, p = .937). A picture of the Activity Monitor final data analysis screen for the "dark" NIR lighting condition is shown in Fig. 5. Fig. 6 shows the video output of our system during an intermediary scene of the same trial; the current location of the rodent is indicated by the crosshair, with the small dots showing previous locations of the rodent.

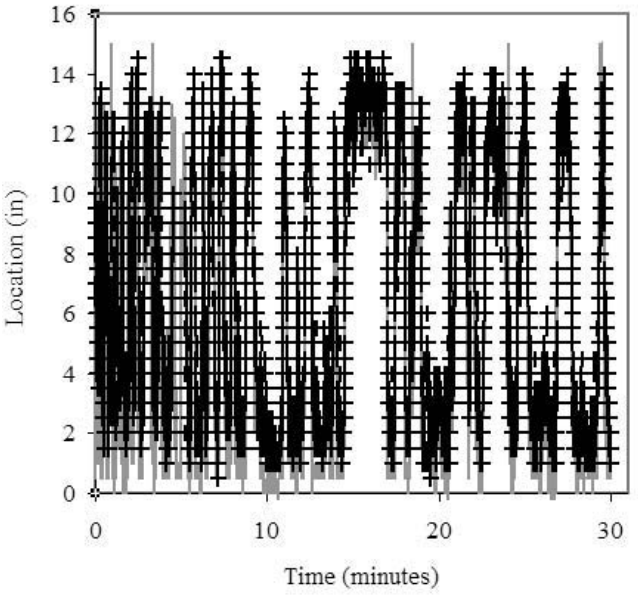

Fig. 4. "dark" condition illumination results – beam-break output indicated by gray lines, video tracker output by black crosses.

The location and confirmation algorithms used here improve the results for this application from those of [9] because it can track a rodent that is a similar color to the background (as in Fig. 5). It also is still computationally simple, and the system is capable of running faster by eliminating the need to perform calculations if the rodent is not moving. Therefore, the combination of color thresholding and motion analysis proved to be an economical, fast and effective for monitoring the location of rodents in an open field environment.

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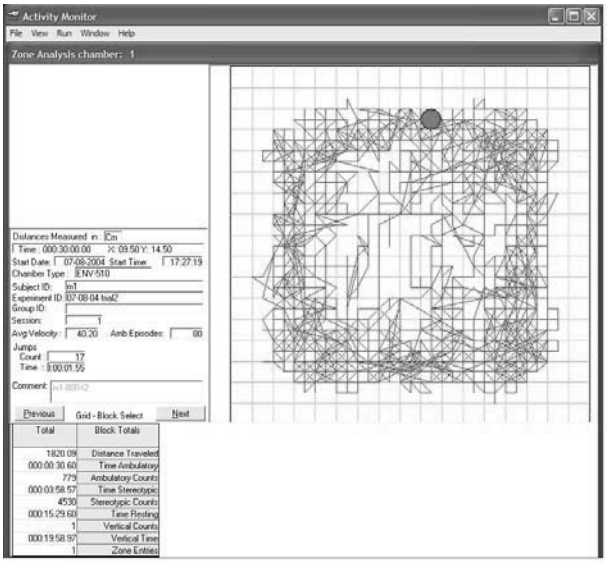

Fig. 5. Activity Monitor beam output for "dark" condition

Fig. 6. Video output of our system for "dark" condition

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