

Computationally-Efficient Image-Processing Scheme for Unilateral Parkinson's Disease Rodent Model

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ABSTRACT

An automatic animal motion activity measurement system is proposed. In the proposed approach, the input image frames (acquired in real-time by a webcam or retrieved from a pre-saved video file) are processed by a combined Background Subtraction and Region Growing method in order to separate the object of interest from the background. The contours of the extracted object are determined using a chain code algorithm and are used to determine the principal axis of the object. Finally, the change in angle of the principal axis over successive frames is used to evaluate the rotation motion of the object. The feasibility of the proposed method is demonstrated by examining the apomorphine-induced asymmetric rotational motion behavior of male adult Sprague–Dawley rats with nigrostriatal lesions. The motion activity measurement system proposed in this study has the advantages of a simple low-cost setup, the potential for real-time implementation, and an accurate and reliable measurement performance. As such, it provides a useful tool for normative, pharmacological and neurophysiologic animal behavioral activity studies.

Keywords: Parkinson's disease, animal tracking, image processing, real time

具高計算效率影像處理之齧齒目動物單側帕金森氏症行動記錄系統

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摘要

動物神經性病變，例如帕金森氏症，對動物行進影響是醫學研究的重要課題。但是記錄動物行進行為，需要大量的人力且極為耗時。本論文介紹一可自動記錄動物行進軌跡之動物運動行為量測系統。系統藉由影像處理分析即時拍攝之動物行進並記錄運動軌跡，也可依預錄之影片進行分析，再以背景相減及區域成長將所追蹤動物自背景影像分離，分離之動物影像以鍊碼法描述其外型並計算形狀之主軸，最後記錄動物在連續影像中的位移軌跡及主軸角度

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變化以供學者分析。為驗證系統可用性，研究團隊記錄以 Sprague – Dawley 鼠為例，注射阿朴嗎啡於其腦部特定位置，引發不對稱運動，以模擬其黑質紋狀體病變對行動影響；經與人力觀察比對，系統可正確追蹤及紀錄其行進及方向變化。本系統除即時自動追蹤紀錄以節省人力外，系統硬體僅包含攝影設備及電腦，建置成本低廉。本系統可提供研究運動行為受神經生理病變及藥物影響學者一組有用的工具。

關鍵詞：帕金森氏症，動物追蹤，影像處理，即時處理

I . INTRODUCTION

Parkinson's disease (PD) is a progressive disorder [1,2] resulting mainly from a de-generation of the dopaminergic neurons of the nigrostriatal tract of the brain, and characterized by motor impairments such as akinesia, bradykinesia, postural instability, and tremors [3]. 6-Hydroxydopamine (6-OHDA) is a specific neurotoxin for the dopaminergic pathways [4], and is transported into the cells of the nigrostriatal tract using the same membrane transporters as those used for catecholamine. The toxic effects of 6-OHDA have been attributed to the formation of various oxidants and reactive oxygen radicals [5], which cause lipid peroxidation, protein damage, and amino acid modification. On the other hand, when injuries occur on the nervous system, such as stroke [6], bleeding [7], brain trauma [8], subarachnoid hemorrhage [9], spinal cord injury [10], or neurodegeneration [11], most phenotypes of these nervous injuries may impair motor ability of the patients or modeling animals. In the experimental animal models, the behavioral testing or motor activity is applied for evaluating the degree of brain injury or recovery status, especially in brain injuries.

In response to apomorphine administration, rats with unilateral 6-OHDA induced lesions of the nigrostriatal dopaminergic pathway circle to the contralateral side of the lesions. This circular movement model is widely used in pharmacological and neuro-physiologic studies of Parkinson's disease to test and evaluate the striatal dopaminergic activity or dopaminergic neuron survival in substantia nigra. In such studies, the motion activity (rotational behavior) of the rats is examined using either observational methods or automated methods. Observational methods involve the direct open-field monitoring of the rats in their home cage [12-14], and have the advantage that the qualitative

behavioral patterns of the rats can be observed directly. However, observation methods are time consuming and labor intensive. Accordingly, automated methods are commonly preferred.

Automated animal motion activity measuring systems can be broadly classified as either hardware-based or software-based, depending on the particular method used to collect and analyze the data. Hardware-based systems have the advantage of de-skilling the system design. However, they are rather expensive. As a result, various automatic measuring systems have been developed [15-18] and brought to market (e.g., Videomex-V, Auto-track, Columbus Instruments). Most software-based automated animal behavior measuring systems are based on image-processing techniques, such as object segmentation, contour tracking, and so on [19,20].

In general, physical objects can be categorized as either rigid or non-rigid. For rigid objects, the contours do not readily change (e.g., a vehicle or a ball), and hence their images are more easily processed using automated methods. The literature contains various proposals for vehicle segmentation systems [21] or insect-image recognition [22] based on a rigid assumption. In addition, Chang et al. [23] proposed an intelligent data fusion system for vehicle collision warning based on a combined vision / GPS sensing approach. However, the segmentation of non-rigid objects, such as humans and animals, poses a significant challenge to automated image-processing methods since the contours of such objects typically change over a sequence of frames. Thus, the segmentation of non-rigid objects generally requires a more complex procedure based on the use of level sets [24,25], boundary-based models [26], or active contour-based models [27-30].

As described above, the asymmetric circling rodent model is widely used to test the

effects of pharmacological compounds on striatal dopaminergic activity. One of the earliest uses of automatic measuring methods to assess the effects of neural damage in mice was that reported by [15], in which both the area of the circles circumscribed by the rat and the number of rotational circles walked were observed. However, the method is based on the path cross of the gravity center, which differs from that of the actual rat rotation behavior. Hence significant errors are produced, e.g. ~10% in the case of slow rat rotation. Furthermore, the proposed system requires special hardware and thus has a high cost. In performing behavioral studies based on the asymmetric circling rodent model, the direction of rotation is of interest in addition to the diameter and number of circumscribed circles. However, the literature presently contains very few automated tracing systems capable of providing such an insight.

Accordingly, the present study proposes an automatic system capable not only of recording and analyzing the asymmetric rotational motion activity of a rat, but also determining the direction of rotation. The proposed system has many practical advantages, including a low-cost setup, the potential for real-time implementation, and a reliable and repeatable measurement performance. As a result, it provides a useful tool for a variety of normative, pharmacological and neurophysiologic animal behavioral activity studies.

II. MATERIALS AND METHOD

2.1 Animals

Male adult Sprague-Dawley (SD) rats, aged 8 weeks and weighing 250-300 g, were purchased from the National Laboratory Animal Center, Taiwan. The rats were housed in an experimental animal room with a 12:12 h light-dark cycle and a temperature of $22 \pm 1^\circ\text{C}$. The rats were fed a standard pellet diet and water ad libitum. The study was conducted according to the guidelines of the National Science Council of the Republic of China, Taiwan, and was approved by the Animal Care and Use Committee of Kaohsiung Medical University.

2.2 Surgery

The experiments were conducted between 7:00 AM and 7:00 PM (i.e., during the light cycle). The rats were anaesthetised with chloral hydrate (400 mg/kg, i.p., Sigma) and then placed in a stereotaxic apparatus (Stoeling Instruments, IL, USA). 4 microliter 6-OHDA.Br (1 $\mu\text{g}/\mu\text{l}$, Sigma) or vehicle (sterile normal saline with 0.2% ascorbic acid) was injected into the left medial forebrain bundle (coordinates as A: -2.0 mm, L: 2.0 mm, V: -8.4 mm) at a rate of 1.0 $\mu\text{l}/\text{min}$ for 4 min. The injection was unilateral, with the contralateral structures serving as controls. Following injection, the injection cannula was left in place for 4 min before removal.

4 weeks after surgery, the rats were tested with systemic apomorphine (0.75 mg/kg in sterile normal saline with 0.2% ascorbic acid, i.p.; Sigma, USA) to evaluate the behavioral effect of the nigrostriatal lesion. Specifically, 10 minutes after apomorphine administration (i.p.), the rotational behavior of the rats was evaluated by placing the animals in a black circular cage and then monitoring their movement using the proposed automatic animal motion activity measurement system.

III. SYSTEM DESIGN AND IMPLEMENTATION

3.1 System Architecture and Implementation

Figure 1 shows the basic architecture of the proposed animal motion activity measurement system. As shown, the system comprises four main components, namely Object Search, Object Segmentation, Contour Extraction, and Axis Computation. The input video stream is first processed by the Object Search procedure to identify the region of the input image containing the object of interest (i.e., the rat, in the present case). The Object Segmentation procedure, consisting of a Background Subtraction method [31-33] and a Region Growing method [34,35], is then applied to segment the object from the background.

A variety of foreground and background segmentation algorithms have been proposed in the literature, such as background subtraction, color clustering [36], image-based distance and area measurement [37], etc. Among them, background subtraction is widely adopted due to

its computation efficiency.

The object contour is then extracted using a chain code algorithm [38]. Finally, the principal axis of the object is computed from the extracted contour and used to evaluate the change in rotational position of the object from one frame to the next. The details of each component of the proposed motion measurement system are described in the following sub-sections.

3.2 Object Search

Traditional Background Subtraction methods operate at the pixel level over the entire input image, and tend to include significant noise in the extracted results. Consequently, post-processing using some form of filtering technique such as a Median Filter, Average Filter or Morphology Filter [39,40] is generally required. However, these post-processing operation sometimes blur the contours of the segmented object, and hence degrade the performance of downstream processes. Accordingly, in the present study, the input image is first processed using an Object Search algorithm to identify the region of the image containing the object of interest, and the segmentation process is then performed only in this more restricted region of the image. The Object Search algorithm is as follows:

(1) A cross-shape mask ('+') with an assigned scale is set. In practice, the scale of the mask should be carefully assigned since while a larger value results in a more robust noise removal performance, it may also cause some candidate objects of interest to be filtered out. In this study, both vertical scale(VS) and horizontal scale(HS) of the mask are assumed to be 11. In addition, this mask operates from left to right and top to bottom every 11 pixels apart.

(2) The region that can contain the whole mask is assumed as the candidate object. The center location of the mask is marked as O_i , which will serve as the seed point of region growing in the later process. The remaining regions are assumed as noise. Considering the illustrative example shown in Fig. 2, the green regions indicate objects or noises. The pseudo code of the algorithm is as follows:

```

N=1
FOR I=7 to Height step VS
{
  FOR J=7 to Width step HS
  {
    Flag=true;
    FOR m=0 to VS-1 step 1
    {
      If (b (J, I+m) !=1)
      { Flag=false; break m loop}
    }

    If(Flag==true)
    {
      FOR n=0 to HS-1 step 1
      {
        If (b (J+n, I) !=1)
        {Flag=false; break n loop}
      }
       $O_N(x, y) = (J, I)$ 
      N=N+1
    }
  }
}

```

A bitmap $b(x, y)$ consisting of "1" (seed points) and "0" (background) is constructed.

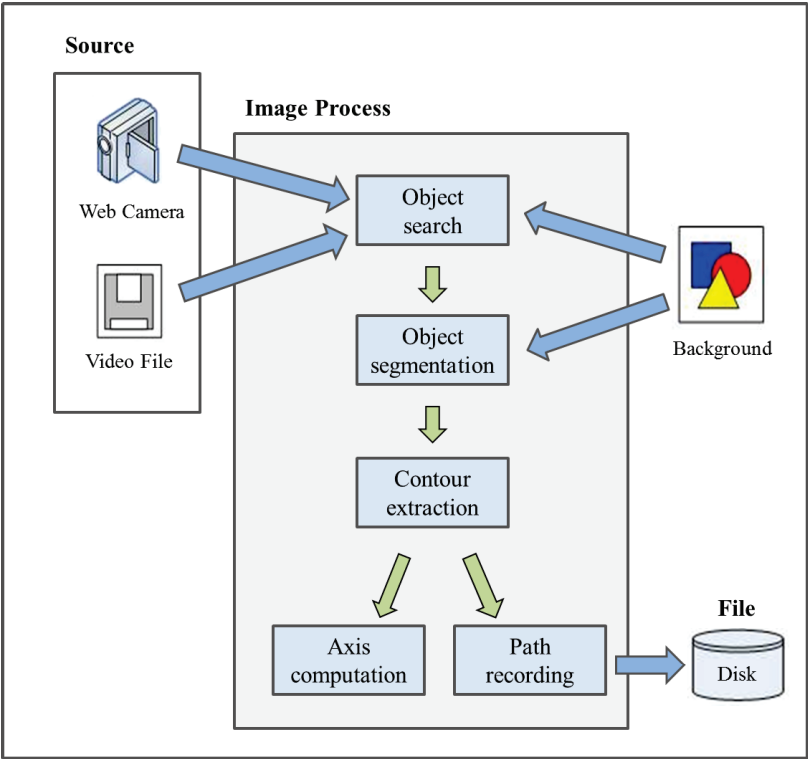


Fig.1. System architecture

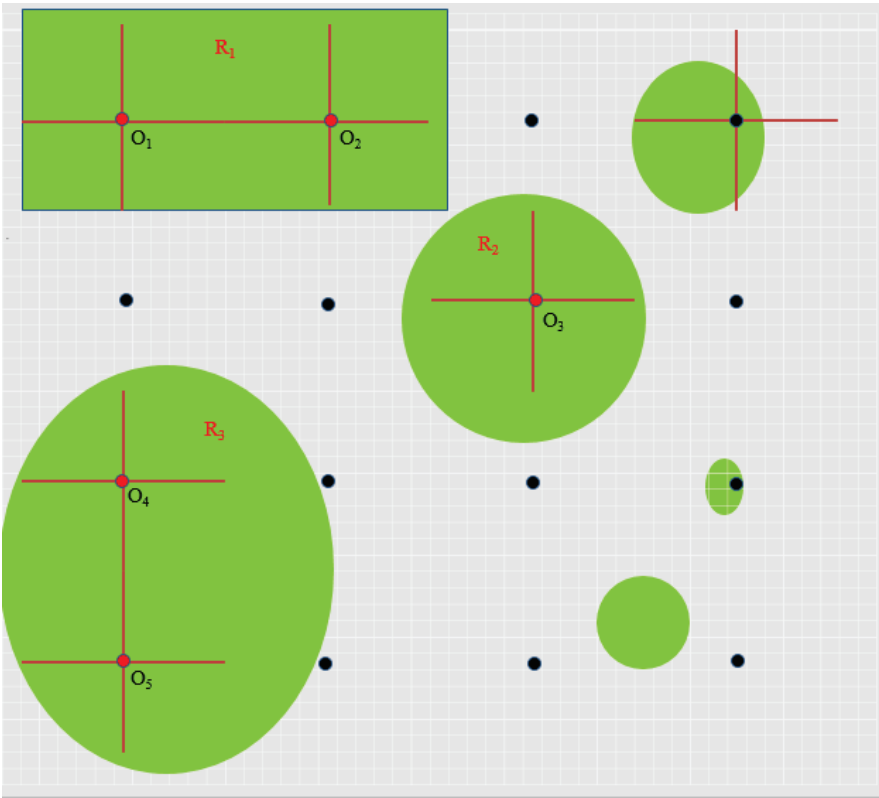


Fig.2. Object search example (Note that green regions indicate objects or noises. The red points will serve as the seeds of region growing.)

3.3 Object Segmentation

Having identified the candidate seeds, we perform region-growing process from the first seed. During growing, if other seeds are

covered, then those seeds belong to the same object and those seeds won't perform region-growing any more. For example, in Fig. 2, when the region is growing from seed O_1 , O_2 will be covered during the growing process since these two seeds belong to the same region, R1.

New region-growing is performed from the remaining seeds. This procedure continues until all the seeds are performed or covered by some region. These resulting regions are identified as $R \{R_1, R_2, R_3\}$. In this study, only one mouse is tested in the experiment, the largest region is chosen to be the object of interest.

3.4 Contour Extraction

Once the object of interest has been segmented, the contour of the segmented object is then extracted using the chain code algorithm. Once the contour is extracted, all of the pixels within the contour are set to "1" in order to fill any intra-object gaps, as shown in Fig. 3.

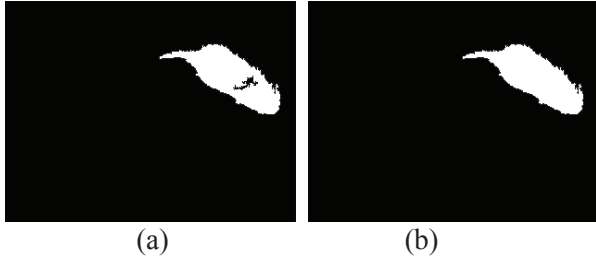


Fig. 3. Filling of intra-object gaps: (a) Segmented image, (b) After contour extraction

3.5 Axis Computation

The binarized image obtained from the chain code algorithm (denoted by the function $f(x, y)$) is processed to calculate the axis of the extracted object. The change in the axis position between successive frames is then used to detect the rotational movement of the object. The axis is calculated using Eq. (1) based on the relative distribution of the pixels within the object. The change in the axis angle, θ , is then calculated using Eq. (2). Note that the coordinates of the object mass center (x_c, y_c) are derived from Eq. (2) in accordance with Eq. (3)

$$M_{p,q} = \sum_x \sum_y x_p y_q f(x, y) \quad (1)$$

$$\theta = \frac{1}{2} \tan^{-1} \left(\frac{2M_{1,1}}{M_{2,0} - M_{0,2}} \right) \quad (2)$$

$$(x_c, y_c) = \left(\frac{M_{1,0}}{M_{0,0}}, \frac{M_{0,1}}{M_{0,0}} \right) \quad (3)$$

3.6 Additional Functions

In addition to measuring the animal motion activity, the proposed system also facilitates the real-time saving to disc of the webcam video file to provide a permanent record of the original laboratory data and to enable subsequent double-checking of the analysis results by alternative methods if desired. In addition, the calculated coordinates of the object mass center are also saved (together with a timing stamp) in order to support further data processing and analysis, e.g., the movement speed and travel distance of the object, the travel track of the object, and so on.

IV. RESULT AND DISCUSSIONS

To demonstrate the computational efficiency of the proposed system, the image-processing algorithm was implemented on a basic PC with limited processing capabilities (i.e., P-4 2.8 GHz with 1 G RAM). The input images were captured using a commercial webcam connected through a USB 2.0 interface. The resolution of the input images was set as 320×240 with 24-bit RGB color. The captured images were processed at a rate of 20 frames per second with a 50% CPU utility. The rats were administered an intracranial injection of 6-OHDA to induce unilateral PD and were then placed (10 minutes later) in the round cage (diameter of 40 cm). Note that a round cage was deliberately chosen in preference to a quadrate cage in order to enable a freer motion of the rats. Nine PD-induced rats were used as observations targets; with each rat repeating the test protocol 5 or 6 times.

As described in Section 3.2, the image-processing algorithm proposed in this study performs an initial Object Search procedure to simplify the subsequent segmentation process. The resulting performance improvement was evaluated by

comparing the segmentation time for three objects of different sizes with that obtained using a traditional Background Subtraction method based on the full image. The segmentation times of the two systems are

shown in Table 1. It is seen that the proposed method reduces the segmentation time by up to 42%. In other words, the potential of the proposed algorithm for real-time implementation is confirmed.

Table 1 Segmentation times of traditional method and proposed method for objects of different sizes

Number of border pixels	Segmentation time for Background Subtraction method	Segmentation time for proposed method	Time saving
203	79 ms	46 ms	42%
321	78 ms	53 ms	32%
1023	79 ms	63 ms	20%

Table 2 Effect of Median Filter in improving counting performance of proposed system

Manual count	System count without Median Filter	System count with Median Filter	Count error without Median Filter	Count error with Median Filter
50	37	49	26 %	2 %
50	41	50	18 %	0 %
50	40	50	20 %	0 %

As described in Section 3.5, the image-processing algorithm proposed in this study evaluates the rotational behavior of the rat by computing the change in the principal axle angle of the extracted object over successive frames. Thus, compared to existing methods, which count the number of rotation circles by reconstructing the entire travel track, the present study determines the number of rotation circles directly from the computed change in the principal axle angle. Notably, by observing the direction in which the principal axle angle changes, the system is also able to determine the direction of the rotational movement of the rat.

In performing rotational motion, the swing of the rat tail may degrade the accuracy of the axis computation process and thus impair the ability of the system to count the number of rotational circles. Accordingly, in implementing the algorithm, a Median Filter is used to adjust the object image, thereby decreasing tail-swing interference and promoting the system accuracy as a result. Table 2 compares the number of rotational circles counted by the system with and without the Median Filter, respectively, with that observed manually. The results confirm that the filter significantly improves the reliability of the proposed system.

Table 3 Experimental results for apomorphine-induced rotational motion of PD rats

Test number	Manual count	System count	FPS	System accuracy
1	100	99	21	99 %
2	100	99	22	99 %
3	100	99	23	99 %
4	100	99	22	99 %
5	100	100	21	100 %
6	100	99	21	99 %
7	100	97	21	97 %

8	100	98	20	98 %
9	100	97	19	97 %
10	100	99	20	99 %
11	100	99	22	99 %
12	100	98	22	98 %
13	100	99	22	99 %
14	100	99	21	99 %
15	100	100	22	100 %
Average			21.26	98.7 %

As described earlier, the experiments were deliberately performed using a black cage in order to increase the contrast between the background and the SD rats. However, as the experiments continue, the cage tends to be spoiled by urine and feces. Consequently, the contrast diminishes and the likelihood of object axis computation errors increases. Accordingly, in performing the segmentation process, a high threshold value of $T=100$ was deliberately chosen in order to filter out such experimental noise.

Table 3 summarizes the experimental results obtained for the apomorphine-induced rotational motion of the SD rats (Table 3). As shown, the average accuracy of the proposed system is over 98%, as evaluated 15 practical tests. Consequently, the reliability and stability of the proposed automated system is confirmed.

Various systems have been proposed (and commercialized) for the automated measurement of animal motor activity. Generally speaking, these systems rely on the interruption of an infrared beam or the triggering of a touch-sensitive sensor as the animal performs motion [41-44]. However, these systems have a high price (typically greater than USD 20,000) due to their hardware requirements. Moreover, the behavioral mode which can be observed or measured by such systems is limited by their hardware design. By contrast, the system proposed in this study has a cost of less than USD 3,000 and provides the ability to examine a greater range of motional behaviors since the behavioral data are computed from an inspection of the pixel changes between successive images rather than by means of some form of optical or mechanical device. Furthermore, in an alpha test, it was found that the resolution of the proposed

system (more than 320x240) was sufficient to segment even small animals such as ants and crickets from a monotonous background.

The literature contains several proposals for automatic animal motion analysis systems based on tracking the spatial coordinates of multiple markers affixed to the animals' bodies [45]. However, webcam-based marker-free systems such as that proposed in this study have the ability to monitor a greater range of real-time movements of the object animals and to gain improved quantitative measurements of the overall animal movements under a variety of conditions [46,47].

In response to amphetamine administration, rats with unilateral 6-OHDA induced lesions of the nigrostriatal dopaminergic pathway circle to the ipsilateral lesioned side. By contrast, the rats circle to the contralateral side of the lesion when administrated with apomorphine [48]. In addition, the experimental results (Table 3) have shown that the count performance of the proposed system is accurate to within 1.5% of that obtained manually. Finally, the system is based on the RGB color space and therefore avoids the need for color space transformation. In addition, the system utilizes an Object Search algorithm to reduce the computational complexity of the object segmentation process, and applies a chain code algorithm to detect the object contour such that the rotational change of the object can be determined. Consequently, the system has a lower computational complexity than existing methods, and thus has significant potential for real-time implementation.

V. CONCLUSIONS

This study has presented an automated

animal motion activity measurement system based on a webcam and a computationally efficient image processing algorithm. The feasibility of the proposed system has been demonstrated experimentally by measuring the apomorphine-induced asymmetric rotational motion behavior of rats with nigrostriatal lesions. Notably, compared to existing automated methods, the proposed analysis system enables the direction of rotation to be reliably determined. The system has minimal hardware requirements, a high measurement accuracy and reliability, and the potential for real-time implementation. Furthermore, the hardware setup can be easily scaled to accommodate animals of a larger size. Consequently, the proposed system provides an ideal solution for a wide variety of normative, pharmacological and neurophysiologic animal behavioral activity studies.

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