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A Novel Walkway System for Measuring the Spatial Gait Parameters of Rats with Spinal Cord Injury

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Abstract

Changes in locomotion, especially related to spinal issues, can signal health conditions. Analysing spatial gait parameters is crucial for understanding these changes. Rats are frequently used in spine-related gait studies to enhance disease understanding, often with the assistance of complex gait analysis systems. Nevertheless, existing commercial systems for rat gait analysis are costly and lack customization options. This paper presents a new walkway system for measuring rat spatial gait parameters in spinal cord injury (SCI) cases. Thirteen adult female Wistar rats were randomly divided into two groups – uninjured (7) and injured (6). For the injured group, contusion SCI was inflicted on the T9/T10 thoracic area of the spinal cord using the NYU Impactor device. The system analyzed stride length (SL) and stance width (SW) and compared its output with manually obtained values. Reliability was assessed using Pearson correlation and Bland-Altman analysis, showing a high correlation ($r > 0.94$) and insignificant bias in measurements. Moreover, disagreement between the systems was less than 5% of the mean value in over 95% of measured outputs, showcasing the system's reliability in measuring gait parameters in normal and SCI-injured rats. Furthermore, the system's output indicates injury in the injured rats with shorter SL and larger SW compared to uninjured ones, demonstrating its capability. This system provides a low-cost setup that can be effectively used to enhance knowledge in studies related to SCI using rats as the model.

1. Introduction

The motivation to commence this research arises from the concern and understanding of the negative impact of spinal cord injuries (SCI) on the quality of life, especially for Malaysians. The spinal cord could be injured due to motor vehicle crashes, sport-related incidents and other reasons. SCI is an extremely challenging condition with limited recovery rates to date [1], making it a critical field for further exploration. Once someone suffers from SCI, his mobility will most probably be permanently affected. Furthermore, their families will have to bear some of the consequences, if not all [2]. Motor vehicle accident (MVA) is the main cause of spinal injuries in Malaysia [3], [4], [5]. As a developing country, this mishap has consequences on the socio-economic. Thus, effort in understanding

SCI is important as this knowledge could be used to propose an effective treatment or therapy. In dealing with SCI patients' condition, the understanding of biomechanics helps medical personnel in setting up suitable care. The term 'biomechanics' relates to the study of the motion of living things through applying the science of mechanics [6]. One of the prominent elements in biomechanics is gait or motion analysis, which is best defined as the set of practices to monitor, record, examine and clarify movement patterns [7]. This is a beneficial tool in assessing health conditions because, from the movement pattern or gait, problems that contribute to one's disability during motion can be identified [8]. Changes in locomotion are often associated with diseases or injuries, including SCI [9]. The locomotive changes are quantified through gait analysis, which is applied in preclinical studies to provide quantitative assessment related to clinical assessments in clinics.

Understanding the gait characteristics of patients with SCI can be done using a valid animal model, as long as the animal model can replicate human physiological and pathological processes [10]. Laboratory animals like rodents, dogs, cats and monkeys are reported to be useful to mimic human SCI, making them suitable to be the model for this type of study [11], [12]. Laboratory rodents such as mice and rats remain the most used laboratory species [13]. Among the reasons are easily bred, easily handled during studies, known genetic background, and having similarities to human disease conditions [13], [14].

In terms of the number of animal samples, several studies have established the reliability of their system with only 10 to 20 samples. For instance, it is reported that Junior et al. used 12 female Wistar rats to assess the reliability of ETHOWATCHER, a system they developed that is capable of tracking laboratory animals from video [15]. Similarly, Gravel et al. employed six adult Wistar rats for the validation of a new computer-aided system capable of extracting joint angles from the rats' limbs during treadmill activities [16].

In exploring the gait characteristics of the SCI-injured rat, many techniques have been used in past studies including kinematics analysis from video recordings [17], [18], [19]. Therefore, this study adopts evaluation through video recording as this approach offers great flexibility in post-analysis opportunities. From previous studies and reports, there are commercially available video-based systems to monitor and analyze the behaviour and gait of rats or small animals such as Ethovision XT and CatWalk XT by Noldus. However, procuring and operating these systems are costly. On the other hand, utilising a non-commercial open-source system such as the MouseWalker developed by Columbia University [20], [21] also poses its challenges, which include difficulties in replicating the exact setup, as modifications can lead to variations in the tool's performance and output. Other reasons include a lack of flexibility for the end users in terms of customisation, coding modification or system alteration [9], [22]. Hence, building its own reliable and affordable system is imperative as such a system will benefit the medical community as well as support the national and global agenda.

This study aims to build an inexpensive and reliable system that can be used to effectively quantify and characterise a rat's gait parameters. This study specifically focused on these spatial gait parameters: stride length for left (SL-L) and right hindlimb (SL-R), as well as the stance width (SW). SL quantify the distance between initial contacts of the same paw in one complete stride, while SW measures the distance between the left and right hindlimb during motion [23]. This system and the present study are important, which envision aligning itself with Revitalising the Healthcare System to ensure a Healthy and Productive Nation in the Twelfth Malaysia Plan [24].

2. Method

2.1 System Hardware

The system was designed to collect walking data from healthy and SCI-injured rats and analyze their spatial gait parameters. The main physical setup of this system is called the walkway, as shown in Figures 1(a) and 1(b). The walkway was built using transparent acrylic of 10mm thickness. The dimensions of the walkway were 80mm (width) x 900mm (length) x 80mm (height). The ceiling and floor of the walkway were made thicker to support the rat's weight and the light emitting diodes (LED) strips. Sufficient light inside the floor of the walkway is important so that the system can focus on the rat's paws during analysis, as shown in Figure 1(c). For a clearer contrast between the rat's paws in the video and the background, a dark backdrop was used. The lighting condition inside the walkway is also important during the experiment, where it is found that a dimmer ambience produces a clearer image of the rat's paws.

Together with the walkway, a motion capture system was developed in-house. The system setup employed a GoPro Black 10 from GoPro Inc. The camera is chosen because besides being inexpensive, it is easily controllable wirelessly via a WiFi connection. For calibrating the image, a ruler is placed at the bottom of the walkway. The measurement from the ruler was used to calibrate the distance walked by the rats. A calibrated and fixed camera setup is important to ensure the reliability of the video recording. During the trial, 60 frames-per-second (fps) was chosen for each test because lower speed causes image loss, while higher speed produces excessive images and increases processing time. The workstation used in this research was a personal computer with 11th Gen. Intel (R) Core i7-11800H @ 2.3 GHz, 32GB random access memory (RAM) and NVIDIA GeForce RTX 3070 graphic processing unit (GPU).

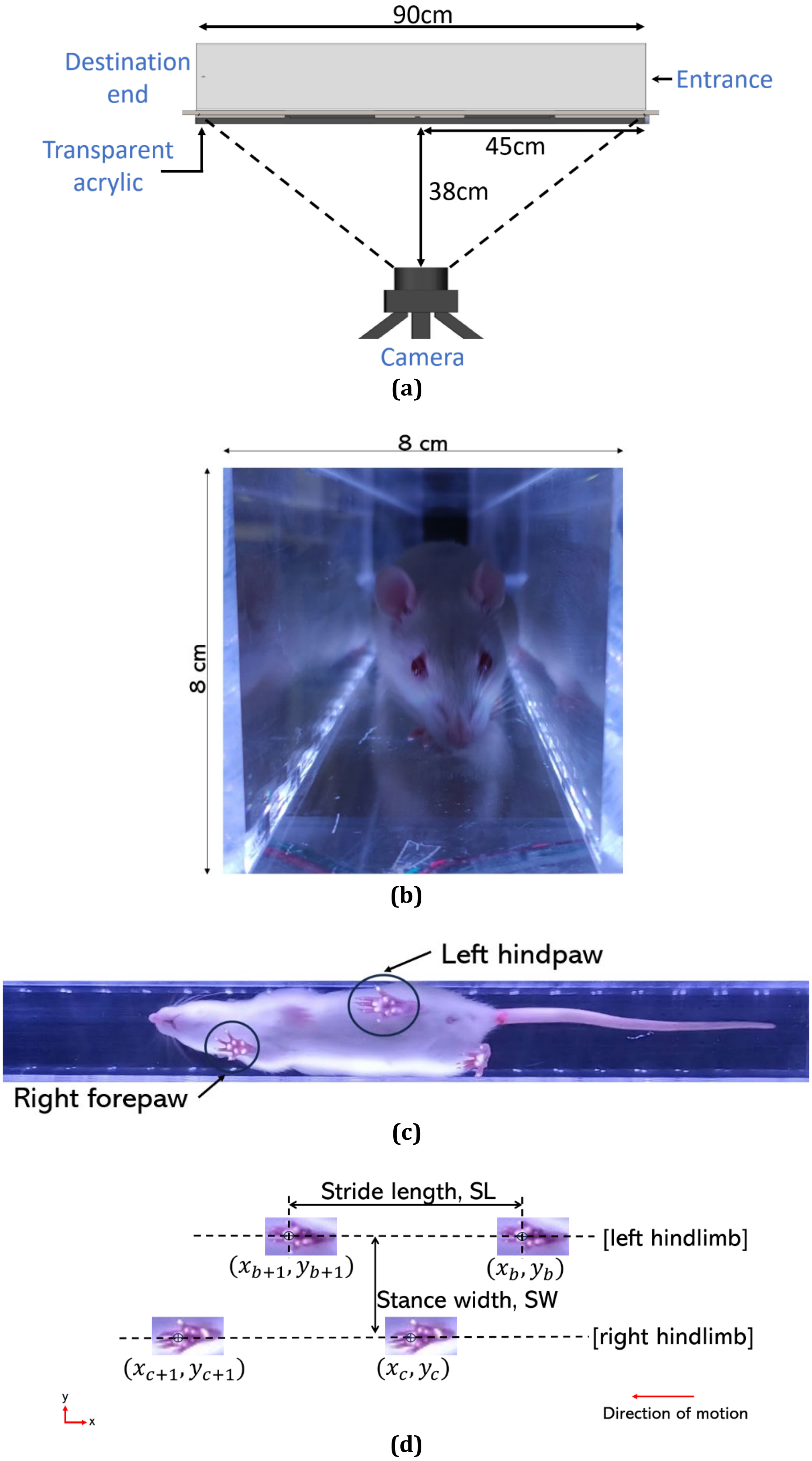


Fig. 1 *Walkway system. (a) System hardware and setup. The camera is placed 0.38m below the walkway's floor for the best view of the 0.9m long walkway. A custom table is used to place the walkway and camera in an evenly horizontal condition; (b) inside view; (c) Bottom view. Every time the paws touch the floor, the light from the LED strip reflects and thus, brightens up the rat's paw. The LED needs to be controlled to produce clear paws images for further analysis; (d) The definition of SL and SW is used to calculate the spatial gait parameters.*

2.2 System Software

The software of the walkway system was developed to determine and analyze the spatial gait parameters; SL-L, SL-R and SW of the rats from video recordings. The recorded videos from the camera were first transferred to the workstation for editing. After being carefully trimmed and edited, the video was sent into the code for analysis. All data presented in this research were prepared using the analysis package, mainly developed using Python. The developed system was based on tracking the paws in two-dimensional (2-D) space and its changes that contribute to variations in spatial gait parameters.

In general, the code is split into three main segments: identifying and tracking the paws, as well as analysing the spatial gait parameters. The trimmed videos were initially processed for paws' marking using DeepLabCut (DLC), a Python-based open-source software package developed by Dr. Mackenzie Mathis and her team. DLC is a tool for marker-less tracking or pose assessment that can perform pose estimation and body parts tracking of living creatures including small animals like [25], [26]. For this study, the DLC software was employed to mark both hindlimbs' paws of the rats in each frame of the recorded video for tracking and analysis purposes. The hindlimbs were selected because they are among the body parts that receive significant impact from the contusion injury, making them worth investigating compared to the forelimbs [27].

After the marking process by DLC, a new set of videos with marked hindlimb paws was generated at the same frame rate as the original videos. To measure SL and SW from the videos, the position of the markers attached to the hindlimb's paws in each of the videos is tracked and analyzed. The threshold value for the color of each paw for the system is identified by the program as shown in Figure 2. In the code, the pixel location of the markers at both the left and right hindlimb of each frame is identified and its pixel positions are recorded. Then, the SL and SW are calculated.

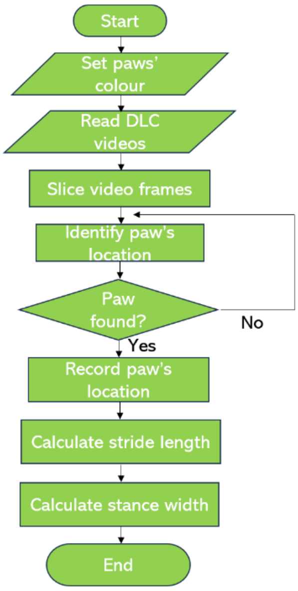
From Figure 1(d), if (x_b,y_b) is the coordinate for the centroid of a left hindlimb's paw at position b, and (x_{b+1},y_{b+1}) is the coordinate for the same paw's subsequent position, then SL of the left hindlimb (SL-L) is calculated as:

$$SL = \sqrt{(x_{b+1} - x_b)^2 + (y_{b+1} - y_b)^2} \qquad (1)$$

Using the coordinate of the right paws' pixel location for the right hindlimb, the same equation is used to calculate the SL of the right hindlimb (SL-R). Moreover, if (x_b,y_b) is the coordinate for the centroid of the left hindlimb's paw at position b, and (x_c,y_c) is the coordinate for the centroid of the right hindlimb at position c, then the stance SW of the hindlimb of the rat is defined as:

$$SW = y_c - y_b \qquad (2)$$

At the end of the output parameters analysis, this research presents its findings on the system's effectiveness in measuring spatial gait parameters for both uninjured and injured rats.



```
Program start
Initialize paw's colour threshold
Read video from DLC
Extract video frames
for frame 1st until frame last
    Find paw
    if rat not found, repeat 'Find paw'
    Record paw's location
Calculate stride length and stance width
Program end
```

Fig. 2 *Program flowchart. Step-by-step sequence to identify and calculate SL and SW from the video recordings. The code can detect the markers' position (x,y) generated by DLC at the hindlimb paws. This information is used to calculate the respective SL and SW*

2.3 Animal Model

To create a contusion spinal cord injury model, Wistar rats were used in this study. A total of thirteen rats (n=13) were used in this study. The current number of samples is acceptable based on past studies [15], [16]. Adult female Wistar rats were weighed approximately 200-250g and randomly subjected into two groups of uninjured (n=7) and injured (n=6). The animals were purchased from the Laboratory Animal Facility and Management (LAFAM) UiTM. All the rats were subjected to surgical procedures according to the approval of UiTM CARE (reference code2/2023/(412/2023). The animals were housed singly in polypropylene cages with corn cobs, where the light was controlled on a 12-hour light and 12-hour dark cycle. The animal was given access to food and water ad libitum.

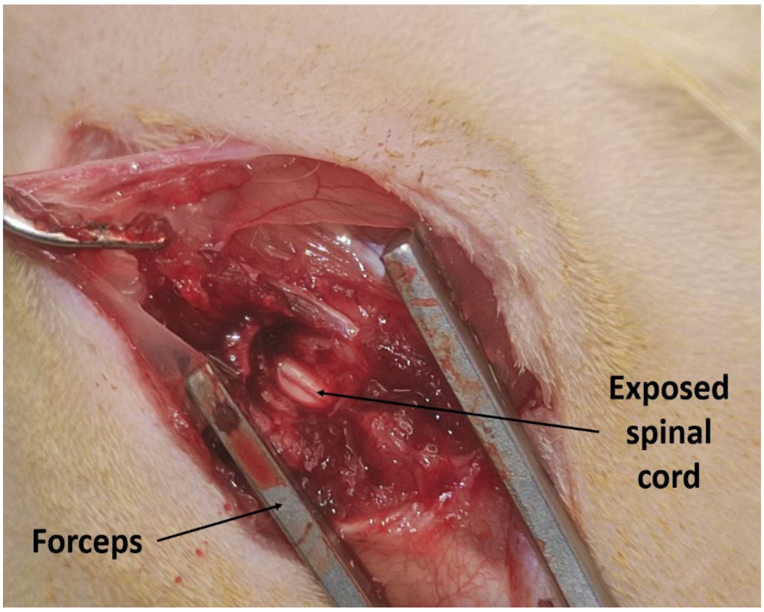
The contusion injury was performed upon completion of laminectomy. The rats were anaesthetized with Ketamine (80 mg/kg) (Troy Laboratories Pty Limited, Australia) and Xylazine (10 mg/kg) (Troy Laboratories Pty Limited, Australia). The skin was shaved at the dorsal midline area and scrubbed with Providone Iodine, 70 % ethanol and Providone Iodine (three times each). An ophthalmic lubricant (Ophtrex Ltd, England) was applied to both eyes of the animal to avoid dehydration during surgery. A skin incision was made along the midline of the dorsum under sterile aseptic techniques. The laminae and transverse processes of T9 and T11 were exposed by gentle blunt dissection of the paravertebral muscles.

In this study, the rod was dropped from a height of 25.0 mm for moderate contusion injury at the T9/T10 thoracic level. Contusion injury was induced on the spinal cord of the rats using the NYU Impactor device (WM Keck Center for Collaborative Neuroscience, USA). The rats were paralysed both on their hindlimb, indicating the surgery's success in inducing contusion injury. Following this, the rats were closely observed for the next 28 days after laminectomy and were humanly euthanized by providing an overdose of carbon dioxide inhalation on the 28th-day post-injury (dpi).

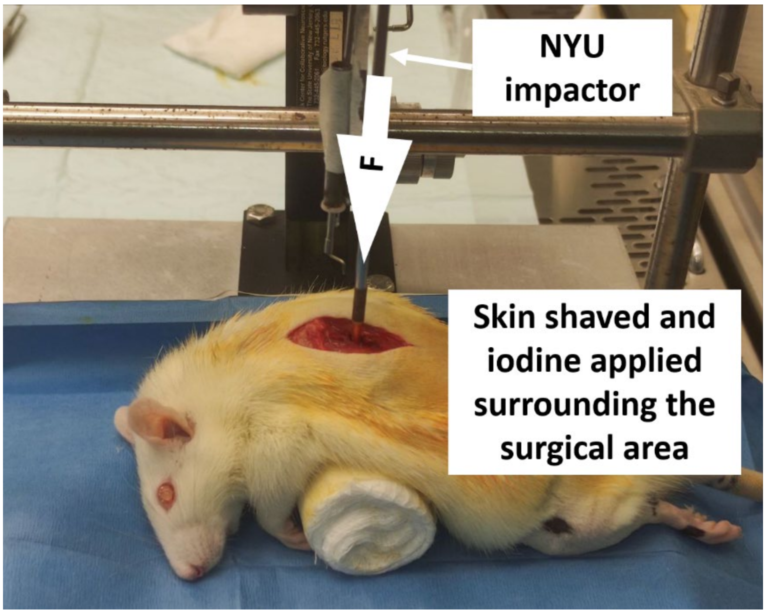
The injury was standardized and ensured to be reproducible across all rats, with each rat experiencing the same impact when the rod (10g in weight) was released from a height of 25mm to the opening of the cord. The rod was elevated and dropped, causing moderate contusion injury. Furthermore, each rat was subjected to the same injury induction conditions, utilising the same equipment setting and performed by the same surgeon as all injury groups.

In addition, the injury site was ensured to remain consistent across all injured rats by strictly following the anatomical landmark procedure described by Medinaceli [28]. Medinaceli demonstrated that the injury site remains consistent across all injured rats, identifying the T9-T11 vertebrae by locating the azygous vein, the prominent vessel inside the multilocular adipose tissue, and the interscapular hibernation gland. This anatomical landmark can be detected by lifting the caudal extremity of the interscapulating hibernating gland from the underlying muscles. The back edge of a scalpel blade was utilised to determine the T5 process, which is positioned beneath the vasculature. Later, the process was counted serially in the caudal direction to T8, T9, T10, and T11. The same approaches were used on all rats in the injury group.

To reduce pain and inflammation after the surgery, the animals were injected with Meloxifam, 1-2 mg/kg (subcutaneous) for 1 day post-op. To keep the animal hydrated for the first two weeks post-op, the animals were injected subcutaneously on both sides of the body with 5cc 0.9% saline (B. Braun, Germany) during less than 24 hours of immediate recovery care. The bladder of the animals was manually expressed 2 times daily or more if necessarily as much as to make sure the bladder was empty. The mashed food (semi-liquid form) was prepared which contained the cocktail of vitamin C, liquid form of normal pellet and clean drinking water that were given independently for a 7-day recovery period. Vitamin C was given to maintain urine acidity and to help boost internal healing after the surgery. The rats were weighed every day for the first week to monitor the food and water intake post-op paradigm. The body of the animals was kept clean and dry in every animal care session to avoid any possible complication of infection. The bedding was changed on frequent occasions especially morning and afternoon rat care for the first two weeks of recovery period. The animals were constantly examined for any excessive bleeding, swelling, respiratory arrest or problem with vocalization during morning and afternoon rat care.



(a)



(b)

Fig. 3 *Images during surgical procedures. (a) The spinal cord is exposed during the laminectomy before contusion injury is induced; (b) Representative image is showing the NYU impactor hitting the exposed thoracic vertebrae at T9/T10. The drop height is set at 25mm from the spinal cord*

2.4 Experimental Procedure

The overall data collection procedure is illustrated in Figure 4, where the preparation involved cleaning the walkway before and after each experiment. Then, the individual rat was released at the entrance of the walkway and let to walk spontaneously inside the walkway to the destination end, where all these motions were recorded from the bottom. The experiment was conducted over four consecutive weeks post-injury before the rats were euthanised, specifically on the 4th (Week 1), 11th (Week 2), 18th (Week 3), and 25th (Week 4) dpi.

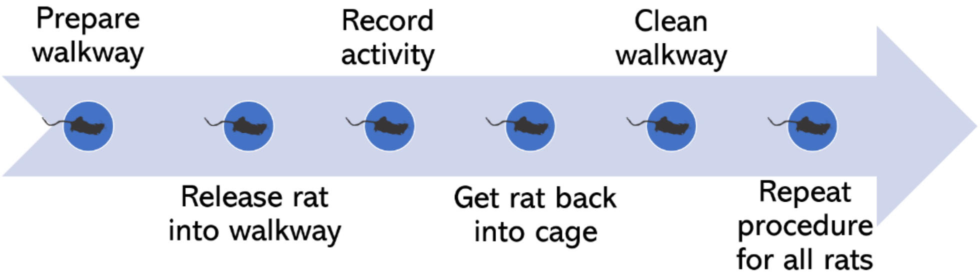


Fig. 4 *Data collection procedure for walkway test. Before putting the rat into the walkway, the equipment must be cleaned to avoid any disruption that can affect the rat's activity, such as the faeces from the previous rat that has used the equipment. When everything is in place, the rat is released freely into the walkway until it reaches the destination end. Their motion in the walkway is fully recorded. At the end, the rat is put back into its respective cages to rest until the next session*

3. Results and Discussion

Rats with SCI were used to validate this newly developed system. To look into the system's capability and reliability, the results during the 4th dpi or Week 1 are presented in this paper. In this study, two outputs are being measured for the uninjured and injured groups of SL and SW. For SL, the outputs are measured for both the left and right hindlimbs (SL-L and SL-R), respectively.

3.1 Reliability

This newly developed measurement system is validated by comparing its output parameters of SL and SW with the parameters calculated from manually marked paws' position. The manual marking is done using ImageJ 1.54d, a Java-based scientific image analysis software developed by the United States National Institutes of Health (NIH) [29]. While the new system automatically detects each paw's position from its colour threshold and calculates the output parameters, manual marking is performed on the relevant frames from the raw recorded videos. This is achieved by carefully selecting the frames for each step made by the rats and identifying the centroid of the paw print in the frame using ImageJ, as shown in Figure 5. The pixel coordinates of the paws' centroid given by the ImageJ are recorded and the subsequent output of SL-L and SL-R as well as the SW for uninjured and injured groups are calculated using the same equation as in equations (1) and (2). There are 89 and 90 frames marked manually for the left and right hindlimb, respectively. On average, each rat generates about six strides per hindlimb, which means they make an average of seven steps in a single test. On the other hand, the distance between the average position of the left hindlimb and right hindlimb for each rat is its SW. When all parameters are determined, the output from the newly developed system is compared with the manually calculated to measure its reliability based on the Pearson Correlation technique and Bland-Altman analysis.

Figure 6(a) shows the Pearson correlation between the output parameters from this newly developed system and the manually marked results for the three parameters of SL-L, SL-R, and SW. The correlation coefficient, r, ranged well from 0.941 and above for all output for both groups, with the SL-R for the injured group being the highest at 0.996. Likewise, Figure 6(b) shows the Bland–Altman plots of the gait parameters obtained by the newly developed system in comparison to the manual computation using ImageJ. Bland–Altman analysis provides a reliable method for assessing concordance and identifying potential bias between two systems under comparison [30], [31]. Each small dot in the plots represents a comparison of value differences and their respective mean for the same output parameters. In the Bland-Altman plots, the x-axis represents the means of the two measurements, while the y-axis denotes their differences.

From the plots, it is seen that the disparities among all tested parameters were primarily situated within the 95% confidence intervals. This demonstrates consistent agreement between all pairs of output parameters calculated using both methods. Table 1 displays the mean values and their 95% lower and upper limits of confidence intervals calculated using a Bland–Altman analysis for SL-L, SL-R and SW parameters for uninjured and SCI-injured rats. The bias is generally small for all the considered parameters, ranging between 0.2383cm and 0.9289cm. On the other hand, the disagreement between the two systems is considerably small. For every output parameter, their dissimilarity was calculated to be less than 5% of its respective mean values for more than 95% of the measured output. Only the output from the SL-L injured group recorded a high number of 11.1% disparity between their difference in measured values from the two methods with 5% of its mean (SL-L uninjured: 2.5%, SL-R uninjured: 2.4%, SW uninjured: 0%, SL-R injured: 2.8%, SW injured: 0%). Overall, the good agreement between both procedures suggests that the newly developed system is an adequate method to measure gait parameters for the uninjured and injured rats in this study.

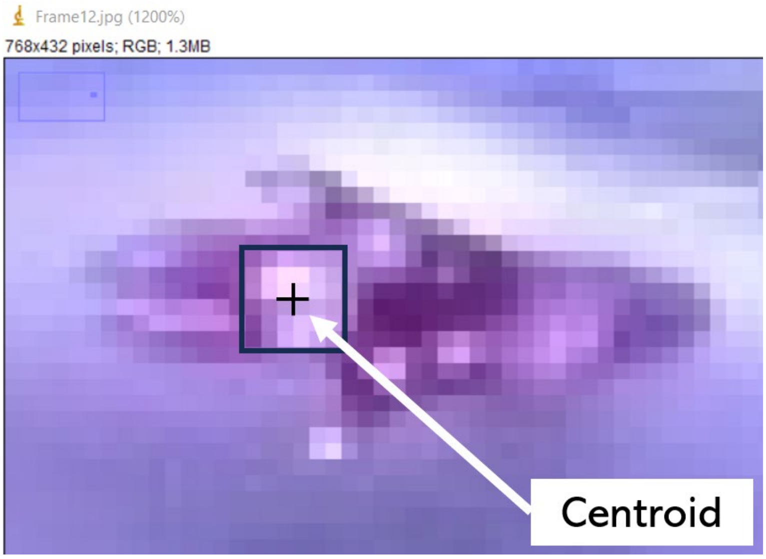
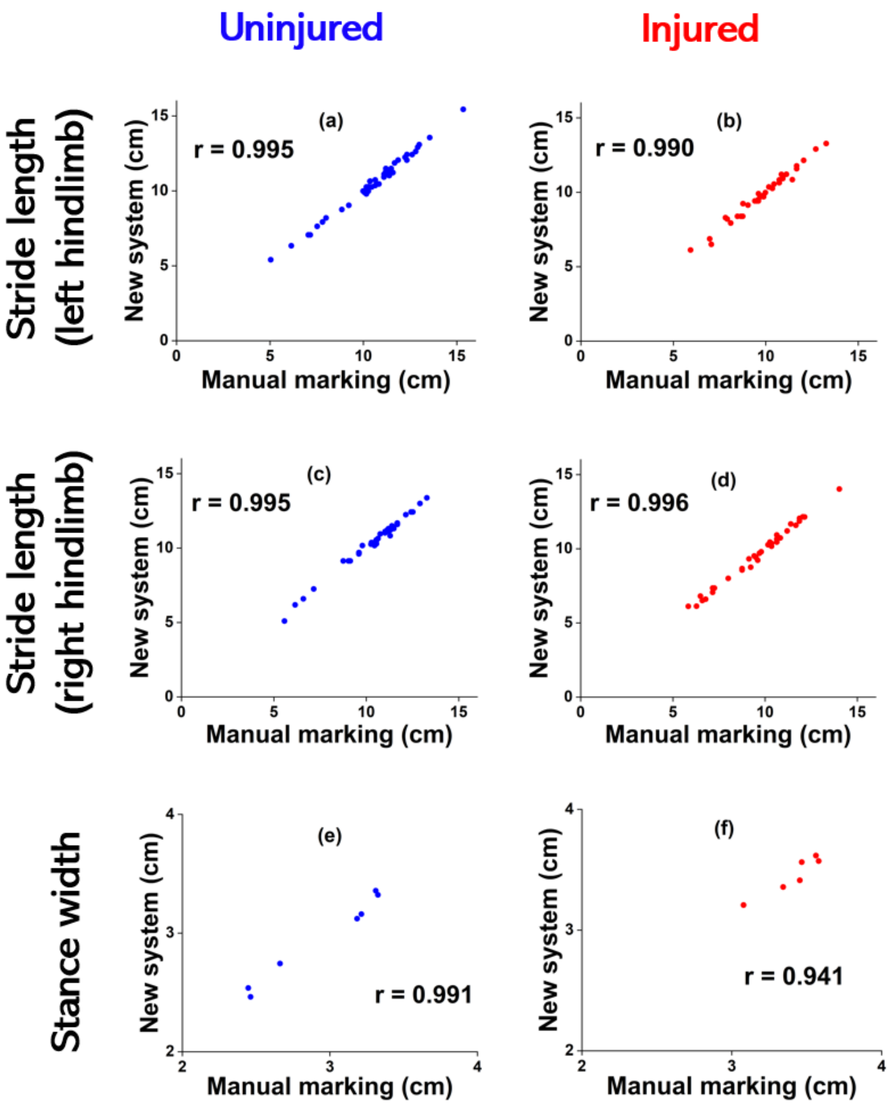
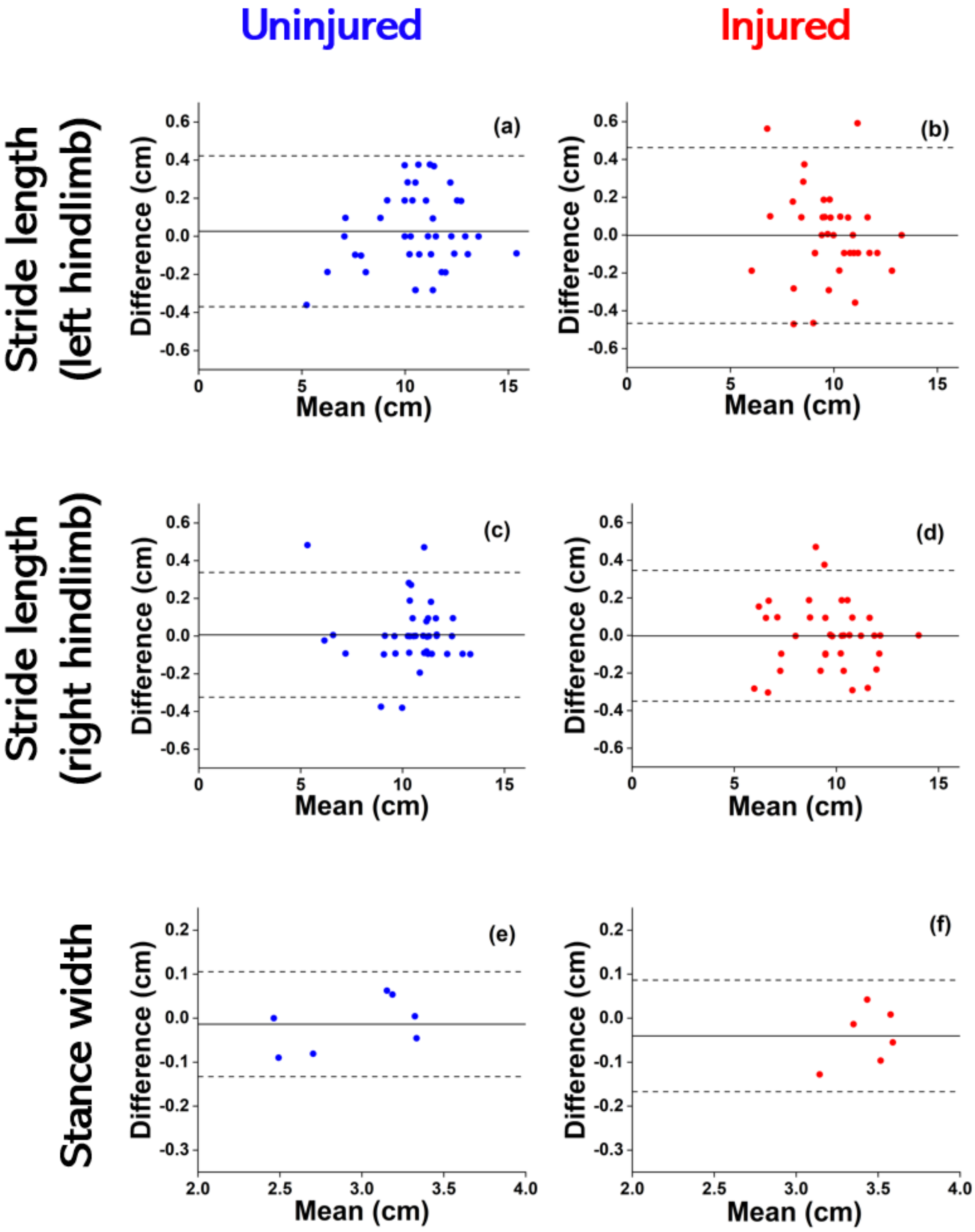


Fig. 5 *Manual marking using ImageJ. This figure shows the paw print of a left hindlimb and how the centroid of the paw is determined manually using ImageJ. The pixel position (x,y) of the centroid is recorded for further analysis*



(a)



(b)

Fig. 6 *Reliability plots of the system. (a) Pearson correlation of the six output parameters obtained by the new system (y-axis) and manual marking (x-axis). The correlation coefficient, r and p values are also highlighted. (b) Bland-Altman plots for the output parameters show good agreement between the two measurement techniques. The dashed lines are the lower and upper confidence intervals of 95%, while the solid line represents the mean of the two systems. It is visible that more than 95% of the data fall within this range, proving the reliability of the newly developed system.*

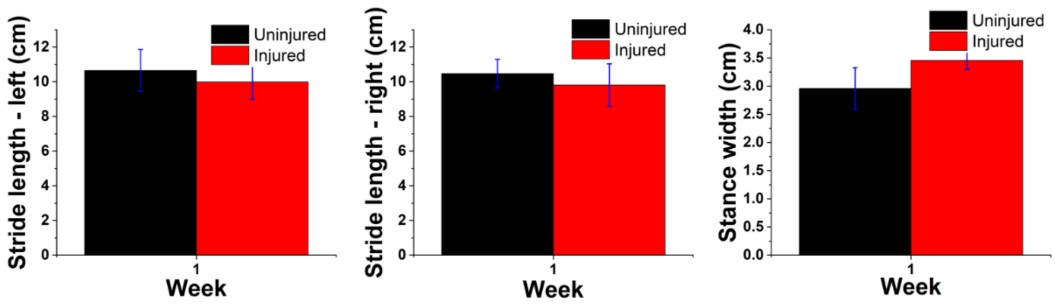
Table 1 *Bland–Altman mean values and 95% limits of lower and upper confidence interval. Lower values of mean or differences indicate higher agreement between the two methods*

Output parameters	Bias	Uninjured	Injured
SL-L	**Mean**	0.0261cm	-0.0015cm
	95% confidence interval	[0.42209cm, -0.3699cm]	[0.4630cm, -0.4659cm]

SL-R	**Mean**	0.0068cm	-0.0018cm
	95% confidence interval	[0.3379cm, -0.3243cm]	[0.3459cm, -0.3496cm]
SW	**Mean**	-0.0135cm	-0.0403cm
	95% confidence interval	[0.1056cm, -0.1327cm]	[0.0865cm, -0.1671cm]

3.2 Results from the Walkway Test

Figure 7 shows the results of the walkway test for Week 1 of the uninjured and injured rats. The SL-L and SL-R in Figures 7(a) and 7(b) show similar patterns, where the SL for the uninjured rats was longer than the injured. For the uninjured rats, their average SL-L and SL-R were 10.655cm and 10.469cm, while for the injured rats, their averages were 9.986cm and 9.808cm SL-L and SL-R, respectively. The shorter SL for the injured rats might be due to the effective SCI sustained by the rats, therefore the present results are in line with a past study [32]. Furthermore, the average values of SW for the uninjured and injured rats were found to be 2.957cm and 3.454cm respectively, as shown in Figure 7(c). The wider stance indicates the presence of SCI. This finding is supported by Hamers et al. (2006) who stated that rats make a wider stance to overcome the instability due to the injury on their spinal cord. The results demonstrate that this system is capable of reliably measuring important spatial gait parameters in both normal and SCI-injured rats. Although only three parameters are presented, by knowing the location of the paws and its other related variables such as the time zone, more analysis could certainly be done.



(a) (b) (c)

Fig. 7 *Results from the walkway test. Average SL of the (a) left; and (b) right hindlimb; and (c) average SW of the hindlimb after SCI. Error bars represent standard deviations. The SL-L and SL-R of the injured rats in Week 1 are shorter than the uninjured rats, while the SW of the injured is larger than the uninjured. This finding indicates the presence of SCI*

4. Conclusion

This research paper presents a newly developed system with a reasonable cost for the automatic measurement of three spatial gait parameters – SL-L, SL-R and SW for uninjured and contusion SCI-injured rats, employing a walkway set up with its motion capture system. Based on the Pearson correlation and Bland-Altman analysis, this newly developed system can be deemed reliable. In addition, the system has successfully measured and assessed spatial gait parameters, SL-L, SL-R, and SW of both uninjured and injured rats. There are clear indications of SCI, with shorter SL and longer SW measured in the injured rats compared to the uninjured group.

It is noteworthy that the system also has potential for future enhancement as its working principles are not limited by rodent size or shape. Furthermore, the setup is easily assembled and utilised to assess spatial gait parameters for a broader range of diseases and injury models. In addition, the coding modification is feasible for end users, allowing more customised parameters and outputs to be extracted. Hopefully, this study will motivate others to explore rigorously the biomechanics of SCI and propose effective treatment and therapy. Finally, the present system could be beneficial to the medical community as well as support the national agenda in improving the quality of life.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of the paper.

Author Contribution

The authors confirm their contribution to the paper as follows: ***study conception and design:*** *Ya'akob Yusof, Mohd Nor Azmi Ab. Patar, Azim Patar, Jamaluddin Mahmud;* ***data collection:*** *Ya'akob Yusof, Azim Patar;* ***analysis and interpretation of results:*** *Ya'akob Yusof, Mohd Nor Azmi Ab. Patar, Azim Patar, Jamaluddin Mahmud;* ***draft manuscript preparation:*** *Ya'akob Yusof, Mohd Nor Azmi Ab. Patar, Azim Patar, Jamaluddin Mahmud. All authors reviewed the results and approved the final version of the manuscript.*

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