Thesis

Design of a Gait Acquisition and Analysis System for Assessing the Recovery in a Classical Murine Model of Parkinson's Disease

Submitted by

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Abstract

Design of a Gait Acquisition and Analysis System for Assessing the Recovery in a Classical Murine Model of Parkinson's Disease

Gait deficits are important clinical symptoms of Parkinson's disease (PD). Data focusing on gait can be used to measure recovery of motor impairments in rodents with systemic dopamine depletion. This thesis presents a design for a gait acquisition and analysis system able to capture paw strikes of a mouse, extract their positions and timing data, and report quantitative gait metrics to the operator. These metrics can then be used to evaluate the gait changes in mice. This work presents the design evaluation of the system, from initial cellphone captured video concepts through prototyping and testing to the final implementation. The system utilizes a GoPro camera, optimally lit walkway design, image processing techniques to capture footfalls, and algorithms for their quantitative assessment. The results gained from live animal study with methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced murine model of PD and treated with 1,1-bis(3'-indolyl)-1-(p-chlorophenyl) methane (C-DIM12) are presented, and it is shown how the quantitative measurements can be used to determine healthy, injured, and recovering gait.

KEYWORDS: Mouse Gait, Parkinson's Disease, Object Detection, Image Processing, MPTP, C-DIM 12

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CHAPTER 1

Introduction

1.1. Background

Parkinson's disease (PD) was first described in a paper entitled "An Essay on the Shaking Palsy" in 1817 by Dr. James Parkinson in Ref. [11]. PD is a pervasive motor disorder resulting from depletion of dopamine (DA) in the nigrostriatal region of the central nervous system [12]. Disorders of gait are one of the most common symptoms of PD. Specifically, patients tend to demonstrate a shuffling gait pattern with a shortened stride length and a reduced overall velocity [13]. These changes in gait of PD patients are related to reduction in the dopamine level [14, 15].

Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin being systemically administered to animals for experimental studies of PD [16, 17]. In human and non-human primates, MPTP induces motor impairments identical to those observed in PD [18, 19]. It can induce neuropathological changes that mimic those of idiopathic PD in experimental animals, especially in C57/BL6 mice [20, 21]. MPTP can cause severe depletion in DA content. Abnormalities in parameters of oxidative stress in PD post-mortem brain tissue suggest that this disease is caused by an overproduction of free radicals [22], the same highly reactive tissue damaging compounds that are suspected of mediating MPTP induced DA neurotoxicity [23, 24]. Thus, these similarities provide appealing hints that the MPTP model may lead to important new insights in pathophysiology and treatment of PD.

Previous pharmacokinetic and pharmacodynamic efficacy studies demonstrated that selected analogs 1,1(3'indolyl)-1-(p-substitutedphenyl)methanes (C-DIM) compounds derived from 3,3'-diindolylmethane (DIM), a metabolite of phytochemical indole-3-carbinol, were protective against loss of dopamine neurons in the SNpc following lesioning with MPTP and probenecid [25]. These studies also demonstrated that C-DIM5, C-DIM8 (DIM-C-pPhOH), and C-DIM12 cross the blood-brain-barrier (BBB) and are orally bio-available [25]. Tjalkens et al. have demonstrated that C-DIM compounds can be used as a therapeutic modality for limiting neuronal loss during neurotoxic and neuroinflammatory injury [26]. Thus, C-DIM compounds can be further used as potential treatment in studies involving MPTP-induced PD to track recovery of patients.

There have been many studies where gait analysis is described and applied for assessing movement disorders in PD [27–30]. There has been a growing emphasis on analyzing the gait in animal PD models. Still there are no benchmarks for characterization of gait variability in bilaterally lesioned animal models [31–36]. Fernagut et al. first inrtroduced the manual measurement of stride length using footprints in bilaterally MPTP-induced lesioned C57/BL6 mice [31]. Klein et al. used footprint analysis and ladder testing on rats subjected to unilateral 6-hydroxydopamine (6-OHDA) lesion for analyzing gait patterns and co-ordination between limbs [32]. Chuang el al. evaluated gait patterns in a unilaterally 6-OHDA-induced rat model [37].

Gait analysis can be used to track recovery of mice as well as rats. Generally mice are smaller in size and quicker than rats. The average amount of time spent per complete stride is 260 ms where the front paws spend an average of 160 ms in contact with the ground and rear paws an average of 185 ms. The average healthy mouse has a mass of approximately 30g. It moves at an average speed of 26 cm/s within a range of 14 cm/s to 43 cm/s. The average stride length is 6.76 cm and the average stance width is 2.7 cm. For a normal gait, the rear paw will land just behind where the previous front paw was. Movement of mice is very coordinated, meaning that for every front paw step taken, a rear paw step of alternating side follows. Therefore, alternate-side, forelimb-hindlimb pair move simultaneously [38–40].

1.2. Objective

The objective of the project is to develop a system capable of acquiring and analyzing data of mice gait, and present the data in easily understandable format. The data acquired should be easily archived and can be used to review the experiments in future if needed. The system should be usable for any and all the the experiments needing quantifiable mice gait analysis. The goal is to provide inexpensive alternative to commercial systems already available in market, which can provide state of the art analysis for mice gait, and make all future experiments less tedious.

The functional requirements of the gait analysis system is inspired from three distinct groups; engineering, operational ease, and data reporting. Firstly, the system should be capable of acquiring data at high sample rate and resolution. The minimum resolution is 5 pixels per cm. This is sufficient to identify a footfall but not enough to identify its toes. The ideal resolution is 15 pixels per cm or greater. This will provide enough data to separate digits on each paw from one other. The minimum sample rate is 30 frames per second. This is equivalent to 4.7 frames of data per contact with surface. The ideal sample rate is 62.5 frames per second. This will give 10 frames of data per contact which is more than sufficient. Also, the system should be able to measure different pressure at different locations of paw to analyze weight distribution accurately.

The operational ease requires setting up the data acquisition device in medical lab with live animals using minimal efforts. The device should be physically robust and easy to transport. It should work to full capacity even after repeated use. The device should be tolerant to animal excretion, and be quick and easy to clean. The system must be self contained, with the exception of PC interface. It is required that no preparation of animal should be needed for the device to be used. Training of the animals in advance, though, is acceptable. Lastly, the device should be easily upgradable, as we live in world where technology is rapidly improving.

Data reported to end user must be in easily understandable formats. User interface should not require more than basic knowledge of computer. The data is reported in following gait metrics: run duration, stride length, swing duration, swing speed, stance, step cycle, duty cycle, cadence, base of support, diagonal dual support, three point support, four point support, average area, average intensity, and paw composites. Additional features include graphical comparison of multiple runs by mapping distance vs time plots, and 3-D graphical representation of paw intensities.

1.3. Thesis Overview

This thesis will explore the design progression of camera based gait acquisition and analysis system for lab mice. Bulk of the thesis will discuss design challenges and related solutions resulting in final system design. Main focus of the thesis was developing inexpensive system capable of highly accurate gait analysis.

The very first challenge faced was setting up lighting system for trackway to create sufficient contrast for camera to distinguish between mouse's paws and rest of its body and background. Variety of lighting schemes were tested for the image processing algorithm to reliably identify every single footfall.

The second challenge was developing image processing algorithm that can differentiate the acquired footfalls data into separate paws, and calculate meaningful final gait metrics.

Image processing algorithms needed to work vast amount of video data in timely manner with minimal number of false or missed matches.

The third challenge was designing a easily understandable graphical user interface for end user so that he/she requires very little additional knowledge to be able to use the data analyzing system.

The first chapter of this thesis presents background of experimental study of PD in classical murine model and summary of gait analysis used for such studies. A brief introduction to gait dynamics of healthy mouse is then provided. It is followed by outline of the objective for developing a gait acquisition and analysis system, and synopsis of main challenges faced during its development. Lastly, an overview of thesis chapters is given.

The second chapter is divided into four major parts. The first part describes the research conducted to find information about the existing technologies for gait analysis. This part provides information about the technology implemented by such devices in the market and discusses their advantages and disadvantages. This part is concluded by deciding the optimum way for us to build such device.

The second and third part of the methodology chapter discusses the problems involving choice of hardware for implementing the decided technique. Different hardware parts are analyzed to find out whether they satisfy the required constraints for building a good gait analysis device. Then evolution of the device is explained by describing how functionality of each chosen part is critical for the device. Then different prototypes built along the way are described, and then specifications for final working device are provided.

The fourth part of the chapter describes the image processing algorithms. These include paw detection and classification techniques implemented for gait data acquired using the final design. Also, different gait metrics are listed which are calculated using the spatial and temporal data of the detected paws. The chapter is concluded with the graphical user interface developed for ease of use of end user, and various functions it can perform.

The third chapter presents experimental results for the study conducted on live animals using the final device. Experimental setup required for the study is explained followed by the necessary training required for mice before conducting the actual experiment. Gait data acquired is presented for different groups of mice in the study along with changes in various gait metrics as the study is progressed.

The fourth chapter concludes the thesis by analyzing the gait data acquired and discussing usefulness of quantitative measures for tracking recovery in mice. This is followed by possible implementation of machine learning algorithm for analyzing recovery progress. Then efficiency of the developed algorithms is given based on positive and false detections. Possible refinements for the device are listed to improve the device in the future. The thesis is concluded by giving a final review of the entire gait acquisition and analysis system.

CHAPTER 2

Methodology

2.1. Survey of Technologies

2.1.1. **Introduction**.

The goal of this thesis was creating a gait acquisition and analysis system which can provide output data which is on par with some of the commercial systems already available in market. This chapter will present brief description of some of the major products in market for gait analysis of rodents, as well as the summary of previous research conducted for building such system. The chapter concludes with a discussion by comparing these various systems and their influence on creation of the new device.

2.1.2. **Previous Research**.

Casey Harr of University of Kentucky has presented her work on creation of gait analysis system in her thesis [41]. To begin with the research, it was important to note that, the supposed device wouldn't work with required capacity if the device to detect force and position of footfalls was to be made from either of the resistive touch sensor, capacitive touch sensor, acoustic touch sensor, fiber optic touch sensor, piezoelectric polymer film, Kistler force plate, Fingerworks touchpad or pressure profile system TactArray. The reasons for these not being used are discussed at detail in [41]. To summarize, their respective drawbacks were, lack of resolution, susceptibility to fluids, complexity of the controller to use the sensor, sensor being out of production, lack of multi-point sensing, unavailability of product and lack of custom design capacity. Her research concludes that, the Machine Vision Camera can satisfy all the required constraints.

The final gait acquisition device in [41] was built with a glass trackway for rodent with Machine Vision Camera located ventrally to record the footfalls. Video data is then analyzed using image processing algorithms.

2.1.3. **Tekscan Animal Walkway System**.

Tekscan Animal Walkway System is complete package of hardware and software for detecting and analyzing animal gait [1]. Gait acquisition sensors are built from a combination of force sensing resistors and piezoelectric gait arrays. The sensors are available for customization in both size and resolution. The suggested sensor for detecting mouse gait is a very high resolution walkway model 5101 with a size of 22.4 x 11.2 cm (8.8 x 4.4 in) and 3,872 sensor elements. Available scanning rates are from minimum of 100 Hz upto maximum of 440 Hz.

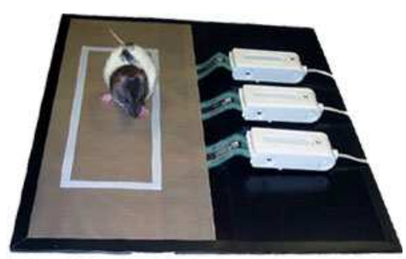

Figure 2.1. Tekscan Animal Walkway System (reprinted from [1])

2.1.4. **Mouse Specifics DigiGait Imaging System**.

Mouse Specifics DigiGait Imaging System is a full gait imaging and analysis device [2]. The system includes treadmill with transparent treadmill belt, digital imaging hardware and software. Camera is located ventrally to capture gait data. The treadmill can be set up for uphill and downhill configurations and variable speeds as per needed. Video is captured at a sampling rate of 150 frames/second. The treadmill belt speed can be varied from 0 to 100 cm/s with a resolution of 0.1 cm/s. After analysis spatial and temporal based 25 indices are reported including stance, swing, braking, propulsion, cadence, step sequence, regularity index, and the sciatic functional index.

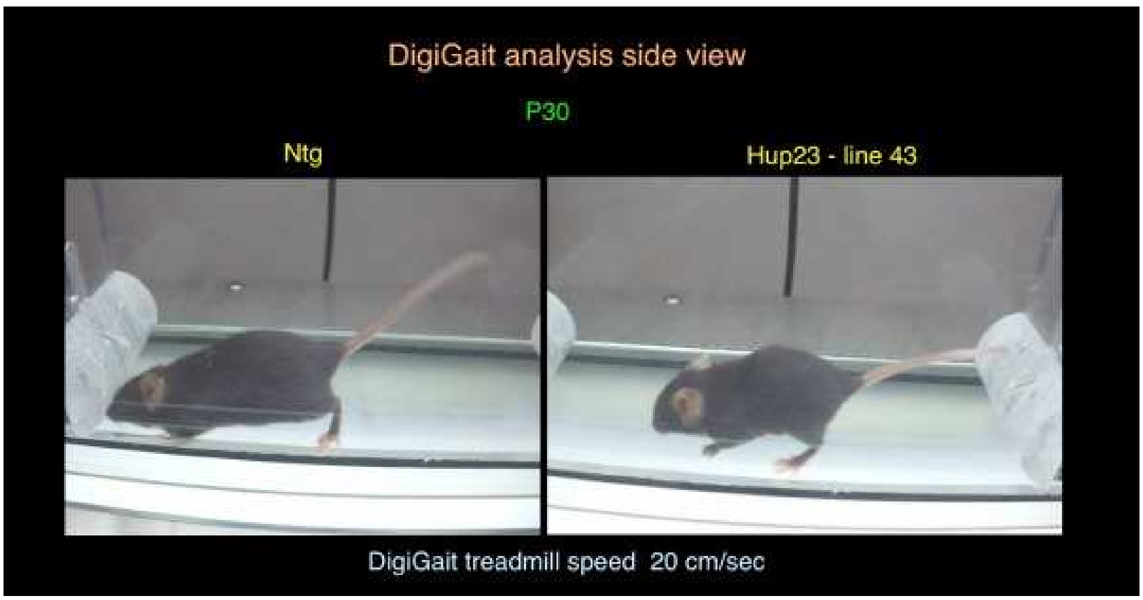

FIGURE 2.2. Digigait Imaging System trackway side view (reprinted from [2])

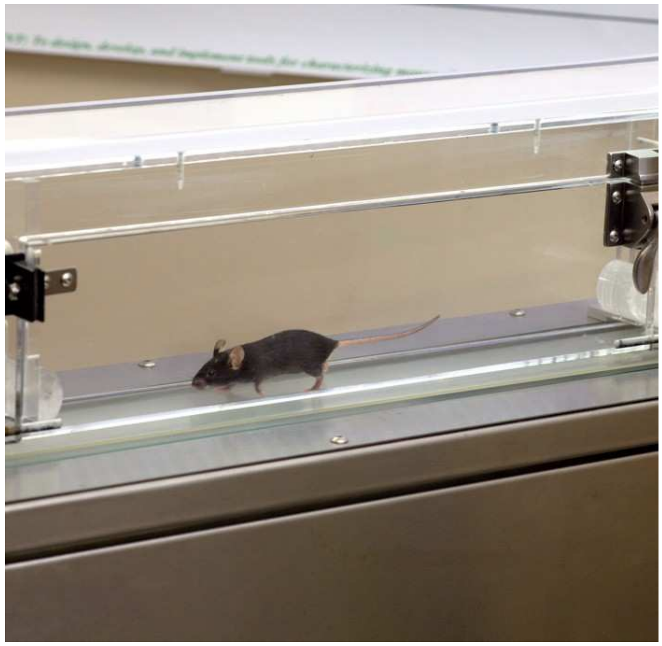

FIGURE 2.3. Digigait Imaging System trackway (reprinted from [2])

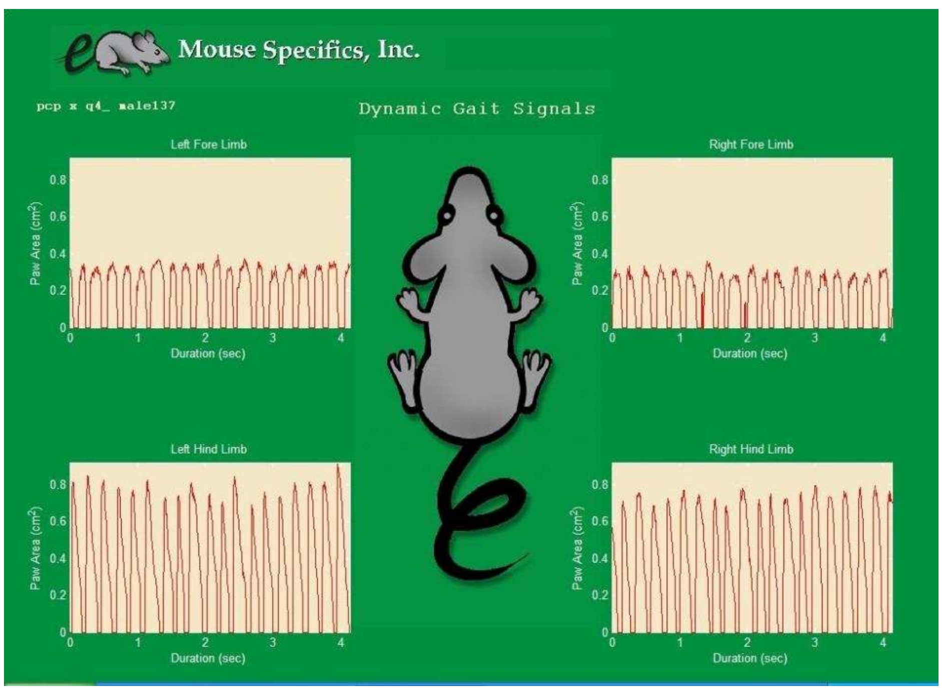

FIGURE 2.4. Digigait Imaging System gait data (reprinted from [2])

2.1.5. **Noldus Catwalk XT**.

Noldus Catwalk XT is gait acquisition and analysis system for mouse gait [3]. The system uses glass trackway and ventrally located camera to capture footfalls. Compartments are created on either side of the trackway to contain animal. The trackway is equipped with adjustable intensity LEDs to create contrast between paw prints of mouse and rest of its body. Recording of video is performed under red ambient light conditions. The footprints are captured at 100Hz with a high speed color camera. Various gait metrics such as print area, brake and propulsion phase duration, intensity, stride length are reported based on spatial and temporal data, and can be labeled as per animal id or group.

Figure 2.5. Noldus Catwalk System side view (reprinted from [3])

FIGURE 2.6. Noldus Catwalk System front view (reprinted from [3])

2.1.6. **Discussion**.

The basic solution for designing any gait acquisition system is having sufficient long trackway on which a single mouse can run across and which has a capacity to detect the position and pressure of footfalls. Several sensor technologies were found to be lacking in one area or other to detect the footfalls satisfactorily according to previous research in the area. The consensus was found to be use of high end camera for gait acquisition. There were several products already available that had similar functionality to the proposed system; but all of these were found to be too costly, lacked some required feature. The Noldus Catwalk XT is the device with most similarities to the final device, but has to be bought as whole system and hence is not customizable as required.

Use of high end camera provides high sampling rate and high resolution both of which are extremely important in collecting gait data. Further research was therefore conducted in determining a suitable camera and designing trackway to create sufficient contrast for gait acquisition.

FIGURE 2.7. iPhone 4 (reprinted from [4])

2.2. Camera Feasibility Experiments

2.2.1. **Introduction**.

Different cameras were tested for capturing the footfall data of mouse. The goal was to use a cheap camera which can capture video at acceptable frame rate and resolution, and is compatible with computer to transfer the captured data to PC platform. This section will discuss the various cameras used throughout the experiments and concludes with discussion of pros and cons for each camera based on actual experiments.

2.2.2. **iPhone 4 Camera**.

The easiest solution was using any decent mobile camera. The very first experiments was conducted using iPhone 4 camera. The camera is 5- megapixel still camera capable of recording HD (720p) videos at upto 30 frames per second [4]. It can capture videos in color and grayscale mode at the above stated resolution. Captured video files can be easily transferred to PC or Mac using the iTunes software via USB 2.0 port. It is a video for Windows compatible device, so nearly every Windows based video capture program will recognize it. Although the camera did not have the highest resolution ,or faster frame rates, it was inexpensive and easy to use for initial testing.

The videos were captured at 30 frames per second at a 360x270 resolution in color mode. That turns out to be nearly four frames per foot contact time. Considering 100 cm trackway length, it turns out to be 3.6 pixels per cm given the video only captures the trackway and nothing else.

2.2.3. **HP Webcam HD-3110**.

The second camera used for experiment was a HP Webcam HD-3110 model. It is capable of recording videos at HD (720p) at upto 30 frames per second [5]. It was capable of auto-focusing. It can capture videos at grayscale as well as color mode. The camera comes with a memory card which can be connected to PC via USB port to transfer the data files to computer. Videos captured are in *.wmv format, so nearly every Windows based video capture program can recognize it. It is a camera which comes into any household category and was cheap as well.

The videos were captured at 30 frames per second at HD (720 x 1080p) resolution. This is equivalent to four frames per foot contact time and 10.8 pixels per cm given 100 cm trackway.

2.2.4. **GoPro HERO 3+ Black**.

The final camera tested was GoPro Hero+ edition. It is capable of recording at Full HD (1080) at upto 60 frames per second. It has capacity to record videos in narrow, medium and ultra-wide screen Field of View (FOV) [6]. The camera has Micro USB port for data transfer as well as microSD memory card. Videos are captured in *.MP4 format, thus playable on any regular Windows video capture program. The camera can be connected to mobile phone via Wi-fi and used for capturing videos. This by far is not the usual household camera. It

FIGURE 2.8. HP Webcam HD-3110 (reprinted from [5])

is therefore little bit costlier than the previous one, but it has higher resolution and faster frame rate.

The videos were captured at 60 frames per second at Full HD (1080 x 1080p) resolution. This is equivalent to 10 frames per foot contact and 19.2 pixels per cm given 100 cm trakcway.

FIGURE 2.9. GoPro HERO 3+ Black (reprinted from [6])

2.2.5. **Discussion**.

After going through the easily available and not so costly cameras in market, decision for choosing camera depended on the priorities for acquiring the data. Basic requirement for good data acquisition system is high spatial resolution, faster frame captures, and an easy interface for data collection. The GoPro HERO 3+ stands out in all the above requirements.

The spatial resolution (or pixels per cm of trackway) is determined by three factors: resolution of camera, distance of the camera from trackway, and field of view (FOV) of the camera lens. By adjusting the distance of the camera from the trackway, we can make sure that the picture captures all the trackway. By choosing different wide FOV available for the GoPro HERO 3+, we can increase spatial resolution of acquired picture. The only problem with using wide FOV is that the picture is taken with some barrel distortion. This problem is discussed in details in the upcoming chapters.

One additional use of this camera is it comes with microSD memory card. So, large data can be stored in one place. Also, the camera does not require any specialized hardware for transmitting data to computer.

The only disadvantage of using camera for data acquisition is we lose the data for force being applied during the footfalls. To collect this data we had to improvise our trackway design as will be discussed in later chapter.

2.3. Gait Acquisition Device Specifications

2.3.1. **Introduction**.

This section details the development of trackway for gait acquisition. Many problems were faced during development phase, and each time we had to come up with some innovative solution to deal with those problems. This section will cover evolution of the design with every problem and its solution.

2.3.2. **System Requirements**.

Considering the average stride length of mouse is 6.5 cm, trackway should be long enough to be able to capture atleast 10 complete strides of each paw. Considering the few strides at the beginning and end of a run will not result is good data set, the trackway should be atleast 80 cm long. Also, the trackway should not be wide enough that mouse can turn back in between a run. Considering average stance width of 2.7 cm, the trackway should not be wide more than 5 cm. This will cause mouse to walk on the trackway freely in only one direction. Also, width should be adjustable to accommodate different test cases.

Material used for trackway should be transparent, so we can capture video from ventrally positioned camera. The material should be robust to animal excretion and should be easily cleaned. The trackway should not distort gait data in any manner. The trackway should be lit appropriately to provide sufficient contrast between mouse's paws and rest of its body and background. The trackway should be tolerant to multiple mouse runs without compromising experiment in any way.

Camera should be able to capture video at 60 frames or faster. This will allow 10 frames for each paw contact with track surface. Spatial resolution of image should be high enough to equivalent atleast 15 pixels per cm. The camera should be easily mounted ventrally to the trackway. Also, distance of the camera from the trackway should be adjustable to determine field of view of lens. There should be enough memory on camera so as to capture multiple run without transferring of data to computer.

Stand must be self-contained, therefore, all the necessary sensors, electronics, lighting, and camera must be housed within the stand. There should be enough space at each end of trackway to easily accommodate mouse. Mouse should be easily loaded and unloaded at specified ends. The stand should be easily transportable. The overall design should be able to ensure uniformity in the captured data.

2.3.3. **Design Overview**.

The device is made up of metal stand on which trackway and camera are mounted. Trackway is made from glass plate on which mouse can run. The trackway is enclosed in with plastic material to restrict mouse running in one direction. On either side of the trackway, panels are created to accommodate mouse before and after a run. The GoPro Hero+ camera is mounted ventral to glass plate at the center. Mount distance from the glass is adjustable. Camera angle is perpendicular to mount rod to capture whole trackway in the picture. The trackway is provided with red LEDs from the enclosure above. Green LEDs are located at the base of the glass. This dual lighting scheme creates sufficient contrast between mouse's paws, rest of its body, and background. Intensity adjustable knob is provided for both red and green LEDs to create a perfect contrast. By using LEDs, only the mouse's paws are illuminated when making contact with glass trackway. This contrast is used for paw detection in image processing algorithm. The whole stand is mounted on wheels to easily move from one place to another.

Camera is connected with android mobile device via Wi-Fi. The GoPro android app is used for selecting resolution and frame rate of video. Also, the app is used for giving commands to camera for capturing videos. Video files are transferred to computer via USB port. Captured videos are analyzed using MATLAB software. Object detection and analysis programs are written specifically for these videos to analyze the mouse's gait. Analyzed data is presented in form of various gait metrics. User can access the videos and analyzed data through specially designed graphical user interface.

2.3.4. **GoPro Hero 3+ Camera**.

2.3.4.1. ***Camera Capabilities***.

The camera weighs 74g (2.6 oz) without housing and 136g (4.8 oz) with housing. Camera's resolution can be adjusted from 720p (1280 x 720 pixels) upto 4k (3840 x 2160 pixels). It is capable of recording videos at 120 fps (frames per second) for 720p, 100 fps for 960p, 60 fps for 1080p, and 15 fps for 4k. Videos are stored in H.264 codec or *.MP4 format. Captured videos have 16:9 aspect ratio. The camera is capable of recording videos in three different field of viev (FOV) modes: narrow, medium and ultra-wide. The camera can be connected via Wi-Fi to remote. Android based mobile device can also be used as remote. With Wi-Fi on, estimated battery life for captureing 1080p videos at 60 fps is 1 hour and 20 minutes. The camera comes with miscoSD class 10 memory card with capacity of upto 64 GB. It comes with Mini USB port which can be used for charging and while connected to computer, and can be used for playback/ file transfer/ charging. The camera requires Mac OS X 10.5 or later / Microsoft Windows Vista, 7 and later for better playback experience [6].

2.3.4.2. ***Remote User Interface***.

The camera comes with a free to download GoPro App for android and apple mobile devices. You can connect your mobile device with camera via Wi-Fi. The application provides full remote control of all camera functions. You can watch of what camera sees with live preview for easy shot-framing. You can select different video recording modes as needed and choose from available field of view options. The application can be used for recording videos from camera with hands-free mode. You can view the captured photos and videos. Also, the application can be used for browsing and deleting files on camera microSD memory card.

The application can used for updating the camera software remotely [7].

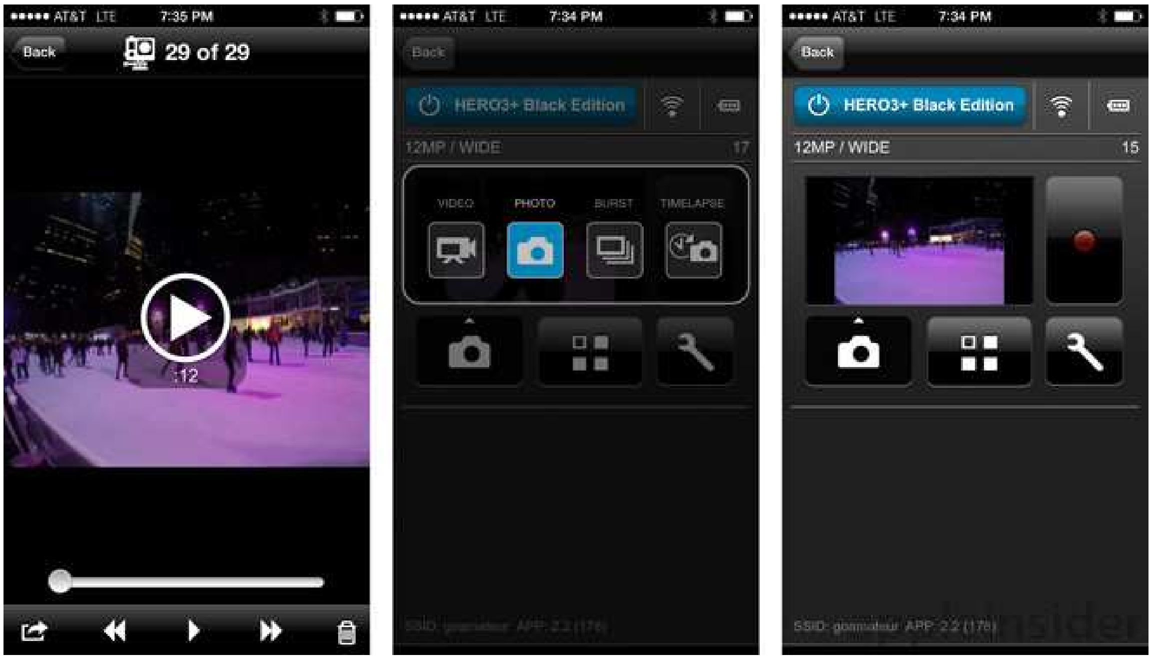

FIGURE 2.10. GoPro Remote User Interface-1 (reprinted from [7])

FIGURE 2.11. GoPro Remote User Interface-2 (reprinted from [7])

2.3.4.3. ***Choosing Field of View.***

Optic Distortion : Distortion is rotationally symmetric image error which increases from the center of the image towards the image margin. In course of this, there is local modification of image scale within the image plane which can be very disturbing for measuring applications. If the magnification rises towards margins of the image field, a square is distorted in a cushion shaped way. In the reverse case we speak of barrel shaped distortion. Wide angle lenses in retrofocus design (back focal length greater than focal length) tend to generate barrel distortion., telephoto lenses (overall length below focal length) tend to generate pincushion distortion [8].

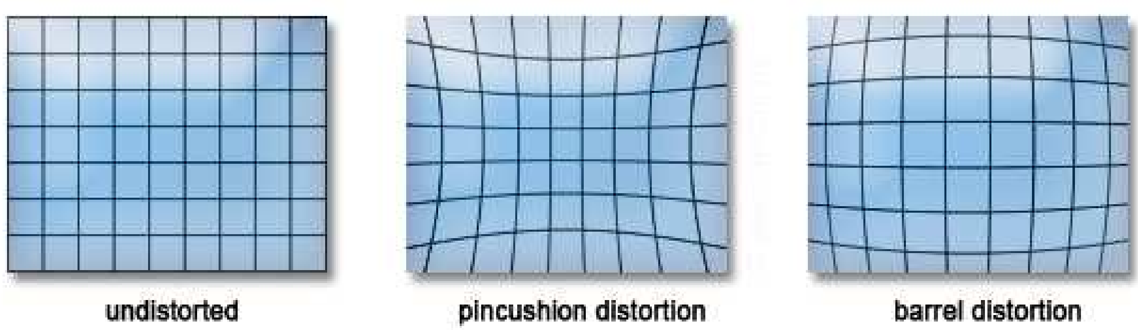

FIGURE 2.12. Types of Image Distortions (reprinted from [8])

Barrel Distortion : When straight lines are curved inwards in shape of barrel, the aberration produced is called "barrel distortion". Commonly seen on wide angle lenses, barrel distortion happens because field of view of the lens is much wider than size of the image sensor and hence it needs to be squeezed to fit. As a result, straight lines are visibly curved inwards, especially towards extreme edges of the frame. Barrel distortion is typically present on most wide angle prime lenses and many zoom lenses with relatively short focal lengths. Amount of distortion can vary, depending on camera to subject distance [42].

Note that lines appear straight at the very center of the frame and only start bending away from center. That is because image is same in the optical axis (i.e. the center of the lens), and its magnification decreases towards the corners.

A non symmetric design of the lens and the aperture before and behind the optic center of the lens leads to image distortions. The image can be curved in barrel-shaped or cushion-shaped way.

The front side aperture assembly of a non-symmetric lens design leads to barrel distortion [8].

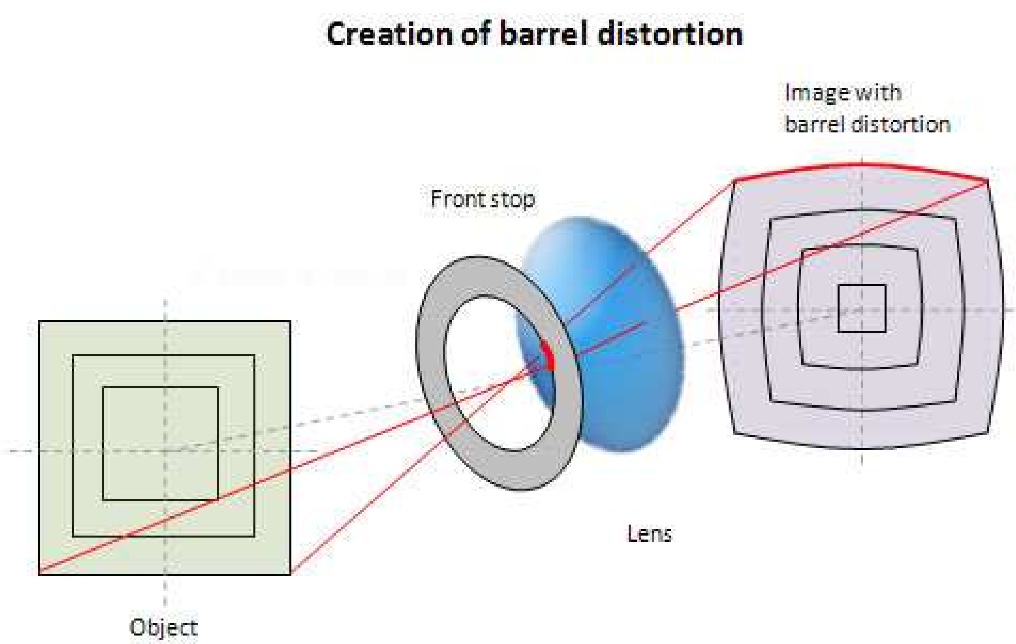

FIGURE 2.13. Creation of Barrel Distortion (reprinted from [8])

The appropriate field of view (FOV) had to be selected to provide greatest area of coverage with shortest working distance and least distortion possible. For the GoPro HERO 3+ camera three different FOV options were available: narrow, medium and ultra-wide. Trackway length of 100 cm was set. Resolution of atleast 15 pixels / cm of trackway was needed to clearly resolve paw digits. All three modes were tested to select the best option for matching all requirements.

The narrow FOV setting needed the camera to be set very close to glass trackway to clearly resolve paw digits. Also, by using using narrow FOV it was not possible to capture whole track and and keep the pixels/cm resolution at satisfactory level. This is because, for covering whole track in narrow FOV mode, distance of camera from trackway needed to be increased. This results in lower pixels/cm resolution.

The ultra-wide FOV setting was able to cover whole trackway while maintaining pixels/cm resolution at high level. Though, the problem with this setting was, even after correction the distortion was not totally eliminated. This is not ideal, as it results in erroneous spatial gait data.

The medium FOV mode best suited for acquiring gait data. Frame captured in medium FOV mode covered whole trackway while maintaining sufficient pixels/cm resolution to clearly distinguish individual paw digits. Also, the distortion was totally eliminated after image processing corrections, hence acquired data was error free. Thus, all videos were taken from medium FOV mode by setting the camera at appropriate distance from trackway as to cover its whole length.

2.3.4.4. ***Limitations***.

The GoPro HERO 3+ camera is highly versatile in several ways. Still it is limited in some aspects. Even-though the camera is capable of recording videos at higher frame rates of 120 fps, resolution available for these frame rates is only 720p. Frame rates are limited to 24 fps for highest resolution of 4k. It is therefore a trade-off between resolution and sampling rate. The ultra-wide and medium mode field of view capture videos with barrel distortion that need to corrected before actual use. This requires extra work while developing image processing algorithm.

Still, it was the best option available which can provide necessary requirements for gait acquisition and these limitation can be worked around to achieve the required results.

2.3.5. **Trackway and Device Hardware**.

2.3.5.1. ***Introduction***.

This subsection will cover evolution of gait acquisition device. A basic gait acquisition device consists of a stand for mounting transparent trackway and camera. The design evolution will discuss the final design of device consisting accommodation for mouse, lighting system developed to illuminate only the paws, trackway enclosure to ensure unidirectional mouse run, adjustable camera mount to determine the distance of camera from trackway.

We will first discuss the physical components of the hardware device. Then we will discuss different models built along the way to final device. Finally, we will discuss various features of the final model and how it ensures that all requirements are met.

2.3.5.2. ***Glass Trackway***.

The initial problem was choosing the appropriate transparent material for building trackway. Two easy choices available were glass and plastic.

There were couple of problems if plastic material was to be chosen. Transparency is generally reduced with increase in thickness of plastic material. So, if the trackway was built thick then it would blur paw prints of mouse. If the trackway was built thin then it would bend at the center where mouse would be walking. To overcome these problems, much costlier investment on plastic material would have been needed. Glass trackway would overcome both of these problems with relatively cheap cost. Because, even if a glass trackway is thick enough to support a mouse, it won't affect visibility of camera. So we can get clear paw prints of mouse from sufficient distance. With appropriate lighting choices a good contrast can be created between mouse's paws, rest of its body, and background.

FIGURE 2.14. Camera Mount

The next task was choosing length of trackway. The average step size of mouse is 6.78 cm. To get any relevant data from a run, a mouse would have to complete atleast 10 full strides of each paw. This will require the trackway to be around 70 cm long. But, initial steps taken during a run do not provide reliable information as mouse is only taking tentative steps. Actual useful gait data is the one when a mouse is walking at normal speed. Hence, 10-15 cm of space was needed on either side of a *good run distance*.

The final trackway is built from glass. It is 2.5 cm thick and 100 cm long.

Also, it was necessary to build some marks along trackway to keep track of distance. It is important for keeping track of available length to pixels ratio. It is also useful for getting actual distance from images with barrel distortion. For this, notches were made into the glass trackway at a fixed distance of 10 cm. Metal filling were used for filling up these notches. These metal filled notches are illuminated green against red background of trackway. These constantly spaced green markers are them utilized in image processing algorithms to convert available pixel values to useful metric of centimeters.

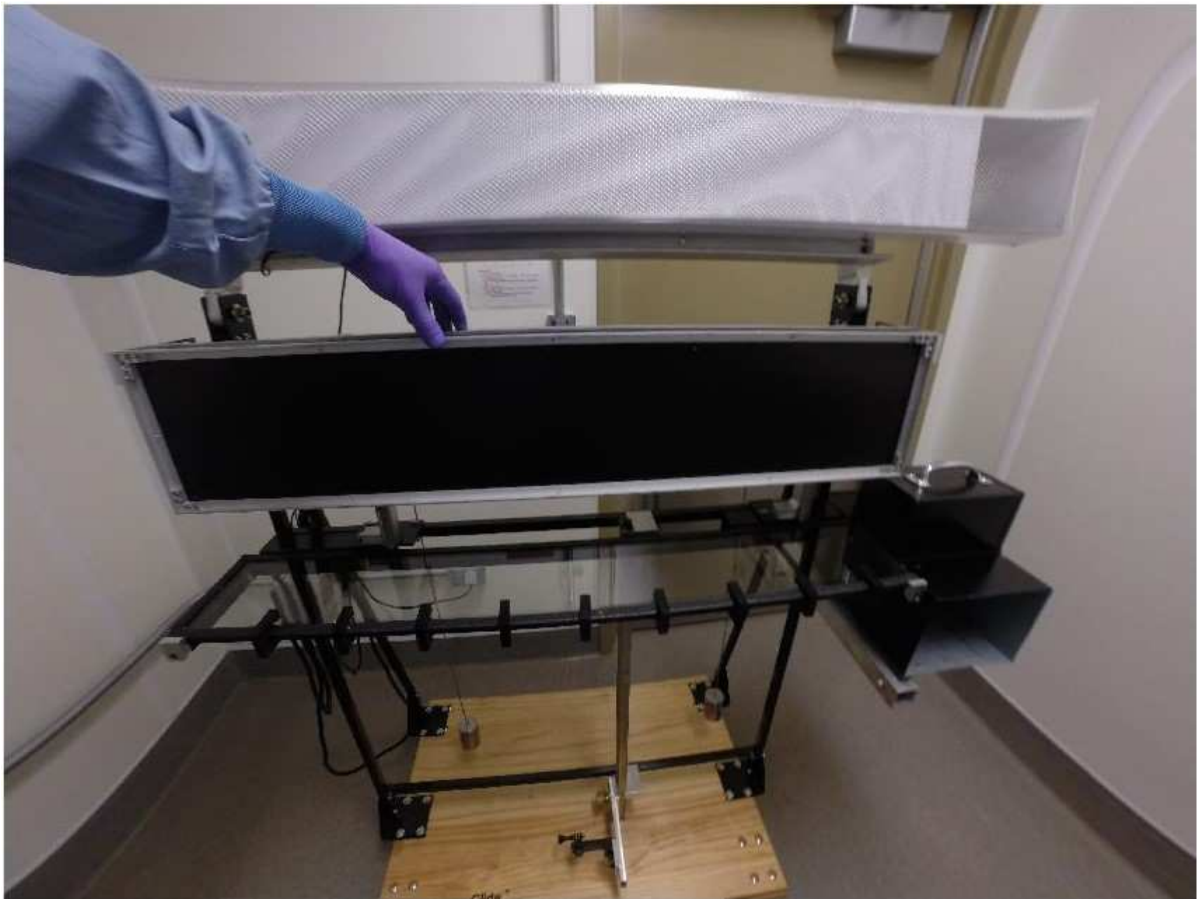

FIGURE 2.15. Glass Trackway

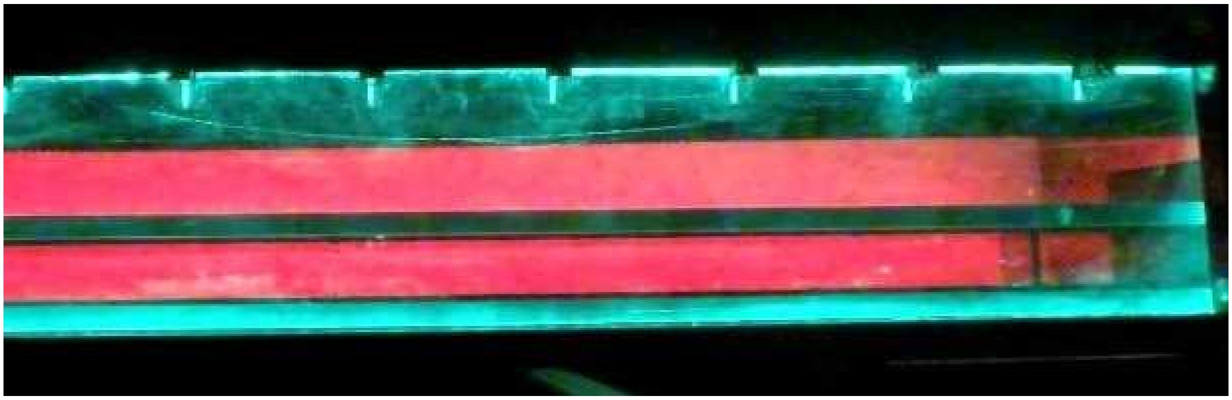

FIGURE 2.16. Equally spaced green markers along glass trackway

FIGURE 2.17. Green LED lit glass plate

2.3.5.3. ***Track Enclosure***.

Rectangular shaped enclosure was made from black plastic material along the trackway. It was necessary to create an effect of mouse running from an illuminated place towards a dark hole to comply with mouse's natural tendency. Therefore the trackway was lit along the way and dark tunnel shaped house was created at unloading end of the trackway.

FIGURE 2.18. Mouse Unloading House

There is a need for creating accommodation for mouse on unloading side of the trackway. The unloading house can hold upto 3 mice at a single time. Also, there is provision for mouse food and water at the unloading area. This way a mouse is driven directly towards the unloading area and gives the second tunnel the effect of a goal at the end of a run for the mouse.

FIGURE 2.19. Rectangular Track Enclosure

Aim of rectangular shaped enclosure along the trackway is to keep the mouse running along the glass trackway. Height of the enclosure is fixed at 10 cm by a removable trap door. Width of the enclosure is adjustable. This is to make sure that once a mouse enters the trackway from the loading side, then it should only run towards the other end of trackway and do not turn back in between a run. The width can be increased or decreased according to size of the mouse.

2.3.5.4. ***Lighting***.

Different lighting techniques were tested to create a good contrast between mouse's paws, rest of its body, and background. If a good contrast is achieved then it becomes easier for the image processing algorithm to detect paws clearly and avoid any false detection or missed detection.

Firstly, ambient light (unlit trackway) was tested. This was sufficient to create contrast between mouse and background but was not enough to create sufficient contrast between paws and rest of the body. This was discarded after only initial few runs.

The next technique tested was applying different lights for background and contact surface. In this way only the part where mouse's paws make contact with glass surface will be illuminated. To create a good contrast two non-mixing lights are necessary. According to color theory, there are three pairs of light wavelengths for whom it is more efficient to record difference between the responses for human eye than recording each type's individual response. These three pairs are : Red versus Green, Blue versus Yellow, and Black versus White. Responses to one color of an opponent channel are antagonistic to those to the other color, and signal outputs from a place on the retina can contain one or the other but not both, for each opponent pair. Hence the pair of lights tested was Red and Green.

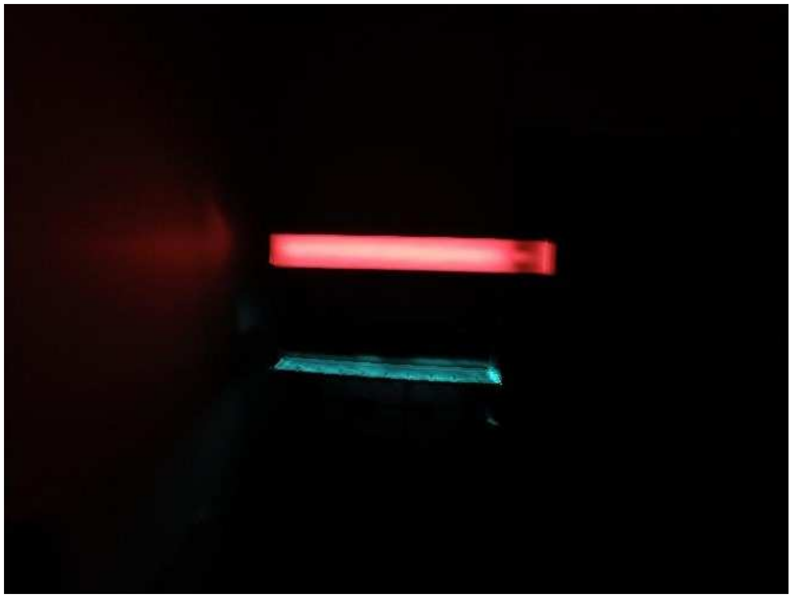

Figure 2.20. Trackway Lighting (Top: Red LED lit track enclosure; Bottom: Green LED lit glass plate)

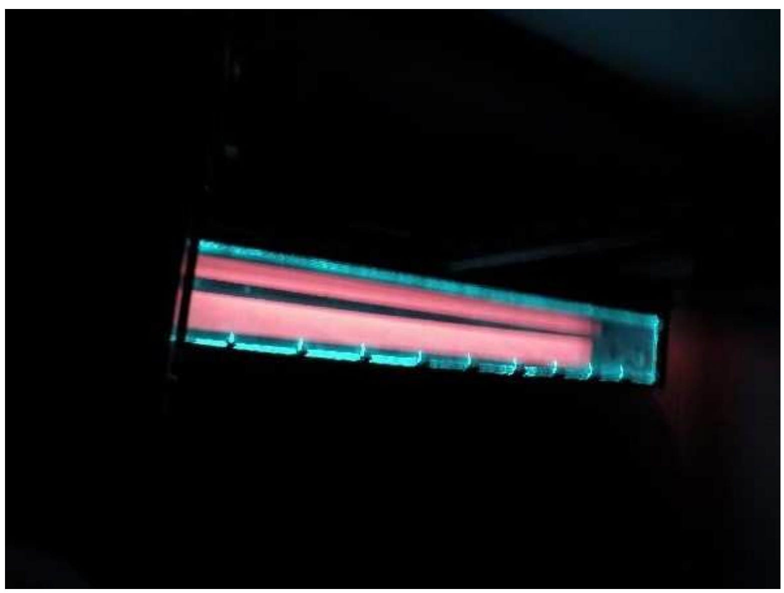

Figure 2.21. Trackway Lighting (Foreground: Green LED lit glass plate; Background: Red LED lit track enclosure)

To create the contrast regular Red light from a hardware store was placed from inside at the top part of trackway enclosure. Green light was flashed at the glass surface from the sideways. This helped in creating sufficient contrast between mouse's paws and body. The part where paws making the contact with the glass surface was illuminated green and the background was maintained red. But using the red light at the center for background created *Intensity Fish-Eye effect*.

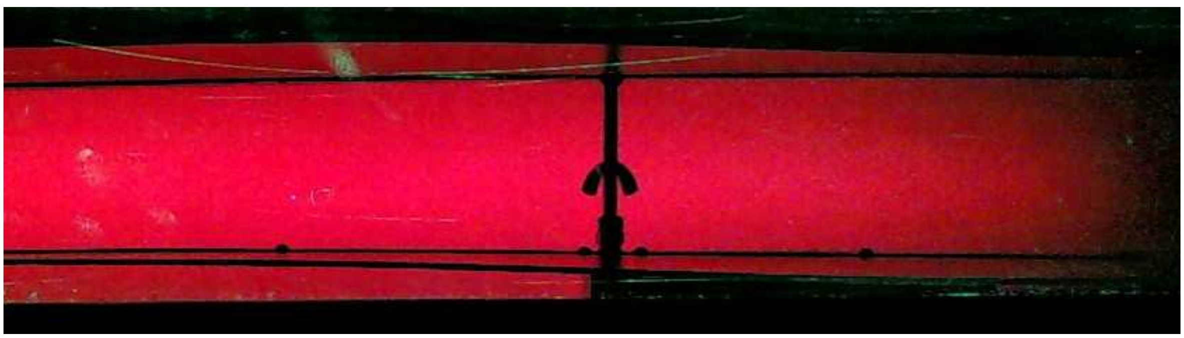

FIGURE 2.22. Fish-Eye Effect created due to center lighting

This means that near the ends of the image there is increased image distortion and variation in light intensity. This was not ideal as it has potential to distort the recorded gait data. But good part of this test was, it gave positive results towards creating good contrast and further efforts were put into testing different technique of using Red and Green lighting scheme.

To create uniform lighting along the trackway, one possible solution was using LED strips along the surface. LED strips of green and red light were purchased from the online shop `www.superbrightleds.com`. These are non-weatherproof flexible 0.5 meter (19.5 inches) light strips with 30 high power 1-chip 3014MD LEDs with adhesive backing. These can be ordered as continuous strip upto 5 meters. These LED strips operate on 12 V power supply and can provide max Lumen of 255.

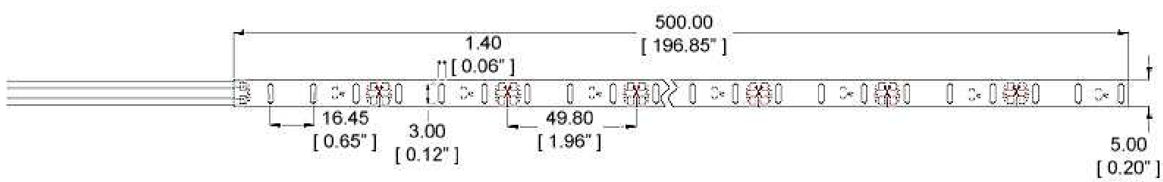

Figure 2.23. LED strip (reprinted from [9])

These strips can be paired with compatible LED dimmers to adjust the light intensity. These strips are 5mm (0.2 inch) wide. From this website Green (wavelength : 525 nm) and Red (wavelength : 626 nm) LED strips [9] were ordered along with LDP 2-A 12 V DC single color LED dimmer capable of dimming LED from 0 to 100%. This dimmer uses Pulse Width Modulation (PWM) knob control for dimming the LED [10].

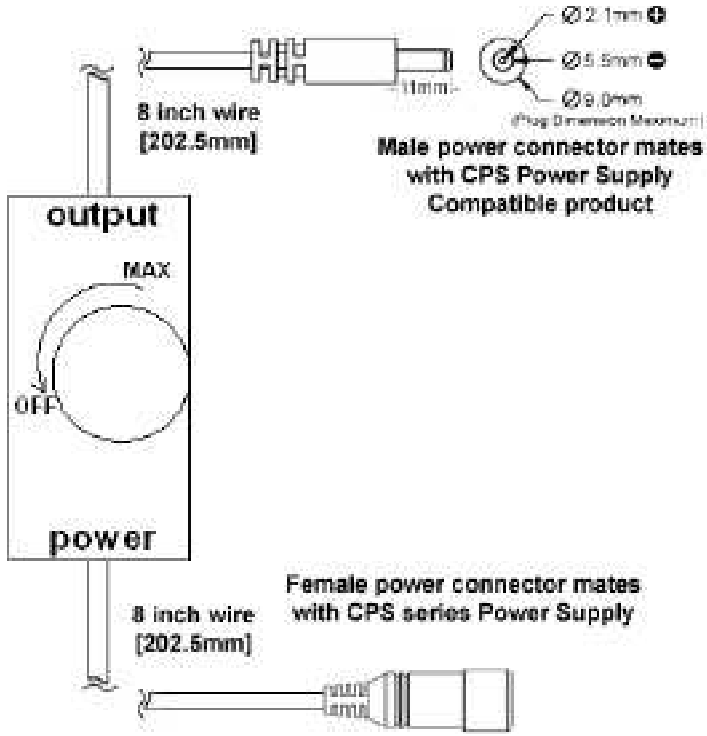

FIGURE 2.24. LDP 2-A LED dimmer (reprinted from [10])

The light provided from the strips was bright and uniform. These LED strips proved to be an excellent solution for the contrast conundrum.

2.3.5.5. ***Prototypes***.

Different designs were built along the way till final device. From basic glass plate as a mouse walkway with ventrally placed cellphone camera till the final design of well lit trackway with GoPro camera, the device went under lot of changes to finally provide solution for all the problems. Below is a brief description of each device built.

Mark I

This was the very first device built. It was just glass plate attached to a metal stand with the cellphone kept underneath to record mouse gait. Cellphone was kept at such a distance that it would capture all of glass track. Videos were captured in ambient lighting conditions. There was no enclosure provided for the trackway. Also, there was no place to accommodate mouse before or after the run.

The cellphone captured videos did not create sufficient contrast between mouse's paws and rest of its body to be detected by image processing algorithm. The mouse kept turning back from where it had started because there wasn't any enclosure for the trackway to keep it bounded in one direction. The camera didn't have sufficient resolution to provide the required minimum of 15 pixels / cm. So differentiating paw digits was impossible.

Mark II

This device was made from properly cut glass plate of required length attached to a metal stand. A mount was fixed at the center of glass which can be adjusted along vertical direction to adjust distance from glass plate. The HP Webcam was attached to the mount and distance was set to capture whole trackway. Trackway was enclosed in plastic casing to limit the space available for mouse. Space was provided at unloading side of the trackway to accommodate mouse after a run. Red light was attached from inside of enclosure and green light was attached horizontal to glass plate to create the contrast for easy detection of mouse's paws.

This device provided many desired improvements from the previous model. Lighting contrast was more than sufficient to detect the mouse's paws. Only the paws were illuminated as they made contact with the glass plate. The problem was centrally fixed light created 'Fish-Eye effect' which distorted the recorded gait data towards end of trackway. The lights did not have any dimmers to improve the contrast as needed. Resolution was improved but still wasn't sufficient. Videos were taken at 30 fps. This provided average of 4.5 frames per contact of paw with glass. This was not sufficient as some footfall were missed or were barely recorded for a mouse walking with a fairly high speed. The glass plate did not have any marking on it to determine pixels per cm automatically.

Mark III

This was the final device which met all the desired conditions for gait acquisition device. This device used same structure as previous model for glass trackway and enclosure along with mouse accommodations and adjustable camera mount. For this model metal filled notches were provided at constant distance of 10 cm along the glass trackway. LED strips with dimmers were used to create an appropriate lighting arrangements. GoPro HERO 3+ camera was used to provide better resolution and faster frame rates.

The mettle filled notches provided useful information for image processing algorithm to determine pixels per cm ratio of the trackway. The full HD (1080p) resolution of camera was enough (18 pixels per cm) to clearly differentiate paw digits. The faster frame rates of 60 fps was very useful as no footfalls were missed while acquiring gait data even for a fast walking mouse. LED strips provided uniform lighting along the trackway and were helpful in getting rid of the 'Fish-Eye effect'. Dimmers provided with LED strips were useful in adjusting red-green contrast to illuminate footfalls more effectively. The only drawback was use of wide lens camera which created barrel distortion for captured images. But this was easily corrected with image processing algorithm with expense of little extra time per video processed.

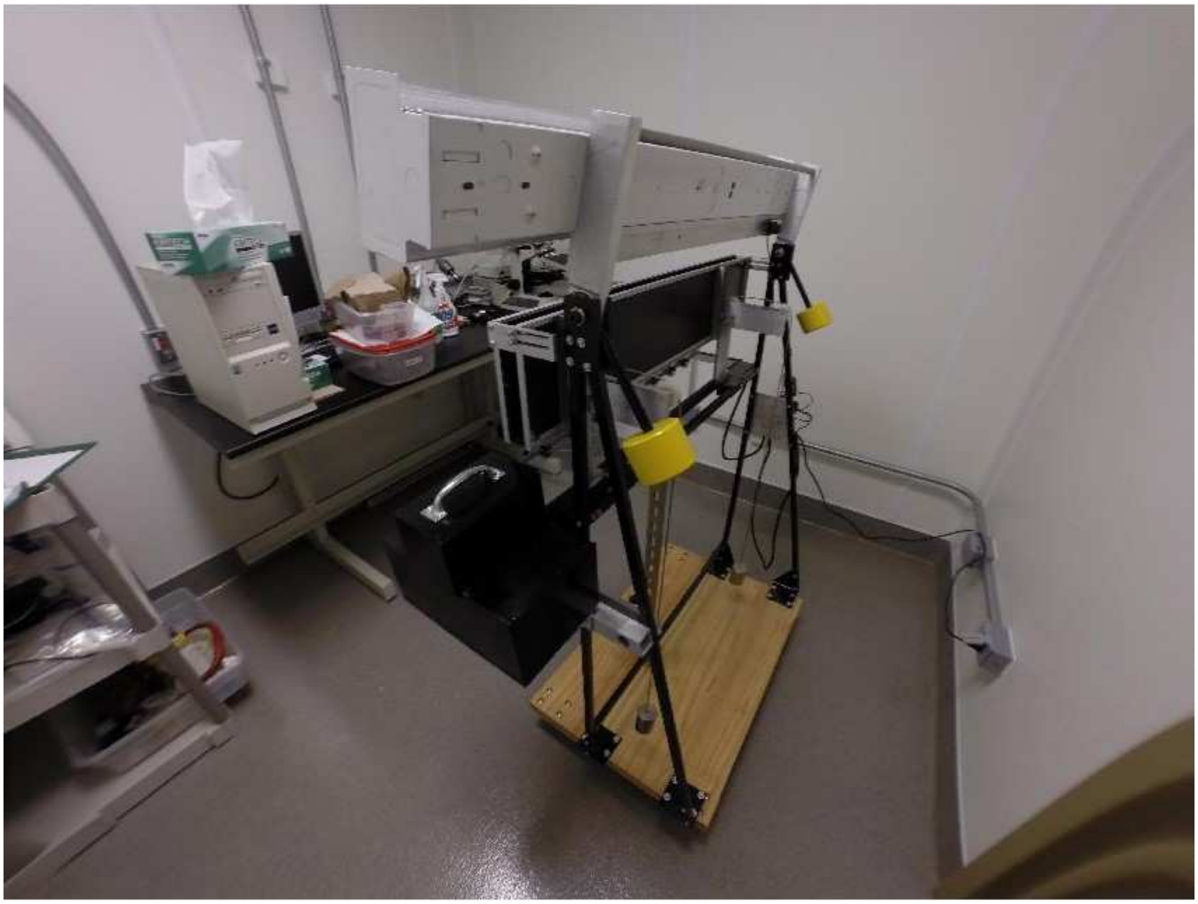

FIGURE 2.25. Fully functioning final gait acquisition device

2.4. Gait Analysis Algorithms

2.4.1. **Introduction**.

Acquisition of gait data is only one half of the task in analyzing gait. To analyze the recorded gait data, various image processing algorithms were implemented. All the image processing algorithms were implemented on MATLAB . Image Processing and Computer Vision System Toolboxes were added to the basic software for easy implementation of image processing techniques. In this section the image processing algorithms used for detection and classification of mouse's paws are discussed along with different metrics calculated for the gait analysis.

2.4.2. **Paw Detection**.

For detecting mouse's paws, it is necessary to differentiate them from rest of its body and background image. This is a major task in the paw detection. If a sufficient contrast is created between mouse's paws and rest of the image then threshold can be used for detecting the paws. The lighting used for gait acquisition helped in creating this contrast.

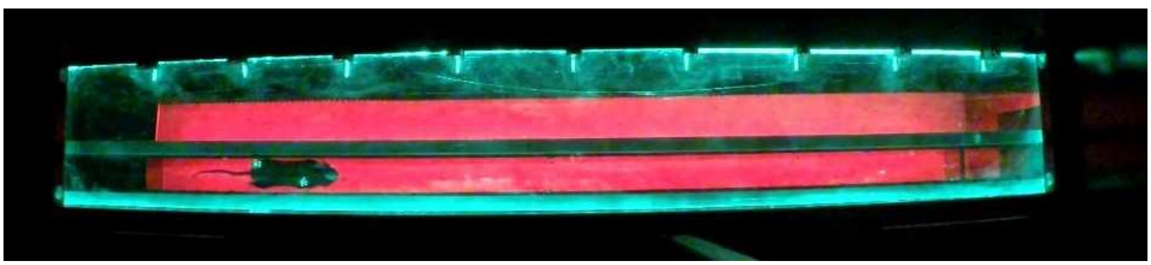

FIGURE 2.26. Original video frame with "barrel distortion"

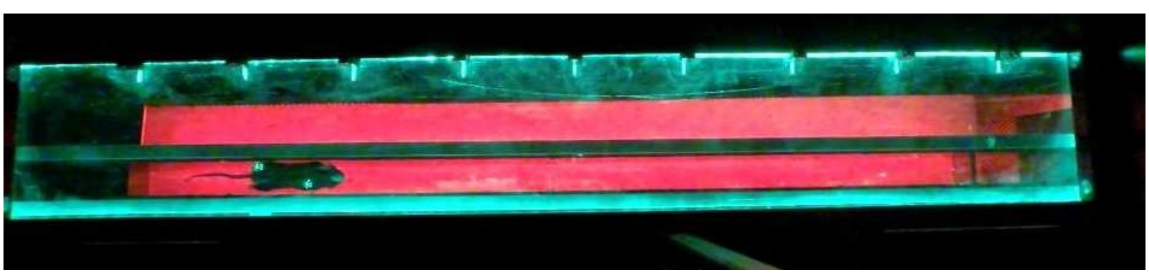

FIGURE 2.27. Video frame after "barrel correction"

For detecting paws, each image in the video is processed individually.

Now, the image looks like figure 2.28 with red background of trackway against illuminated green spots where the paws make contact with glass trackway. A color image records colors in RGB (Red, Green, Blue) format where each pixel in an image is assigned a value for each of these colors from 0 to 255. These three colors serve as primary colors in any color image with RGB format and combination of these colors can create different colors.

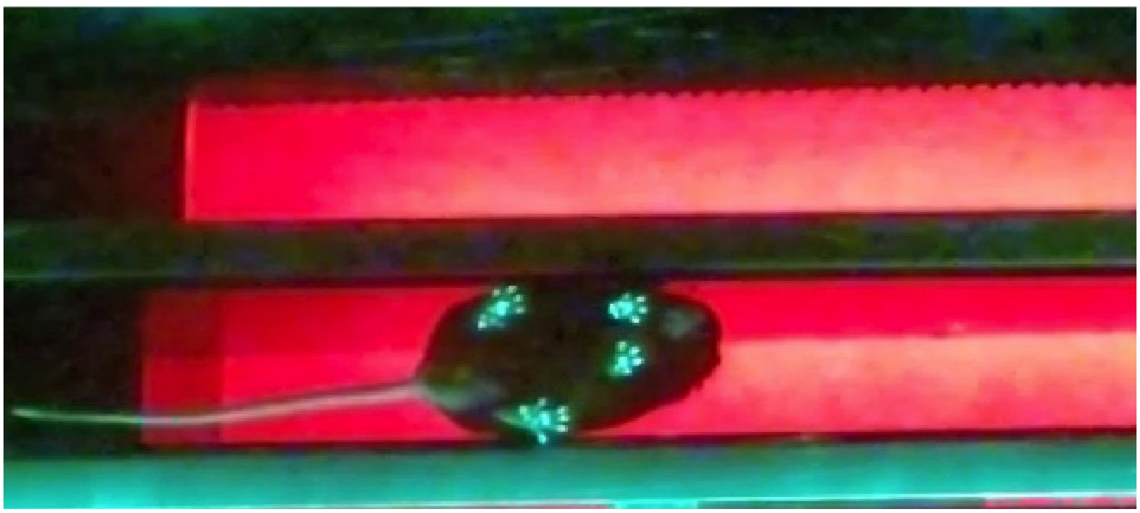

FIGURE 2.28. Zoomed-in trackway showing illuminated footfalls

For the particular application of detecting paws in the images, it is required to detect only the green parts in the image. This is equivalent to detecting the pixels with non-zero G *(Green)* value in the image. But we cannot just label all the pixels with non-zero G value as paws because of noise in the image. To solve this, we use thresholding technique. To separate the paws from the noise value different values of thresholds were tested. The final algorithm was implemented using the threshold value of $Thresh_{Green} > 180$ which eliminated most of the noise while still detecting all the paws.

After performing thresholding for *Green* value, there are still many pixels left in image that needed to be identified as paws. For this clustering technique is used. Clustering is method used to link closely related pixels together to form a larger object. Clusters can be formed by proximity or by connectivity. Proximity clusters are formed by grouping pixels within certain distance from each other. This distance is predefined by some threshold value. Additionally, proximity clustering can be used to form even larger objects by joining the previously formed groups. Proximity clustering is primarily used for joining discontinuous portions of single large object. But use of proximity clustering can result in joining of two different objects within proximity threshold of one another.

The second method of clustering is by connectivity. Connectivity clustering is when one pixel is found and forms a new group, then adjacent pixels get associated with first one, and then pixels adjacent to those become associated with the set. This continues recursively until all the connected pixels are clustered together. The connectivity clustering can become computationally expensive if an image is too large as the algorithm traverses through the image row by row in recursive fashion until no adjacent pixels are left to be grouped together.

Proximity clustering is used for joining digits of paws together which are disjointed from palm area. The threshold distance is set for 15. So any pixels within 15 pixels distance from each other will be grouped together to form a cluster.

After initial clusters are formed, we limit maximum number of clusters to 6. This is done by predefined threshold for size of a cluster. Clusters smaller than certain threshold ($Thresh_{small}$) and clusters bigger than certain threshold ($Thresh_{large}$) are eliminated by this. By performing this elimination, clusters formed by unwanted parts such as noise or dust on glass are eliminated. The maximum number of clusters is decided to be 6 as there is possibility of mouse touching its nose or tail to the glass while walking. The $Thresh_{small}$ was decided to be 30 and $Thresh_{large}$ was decided to be 300 after performing testing with the detected objects.

After finalizing the clusters, centroids of these clusters are recorded for each frame of acquired video. X and Y co-ordinates of the centroids indicate row and column number of the pixels in the images.

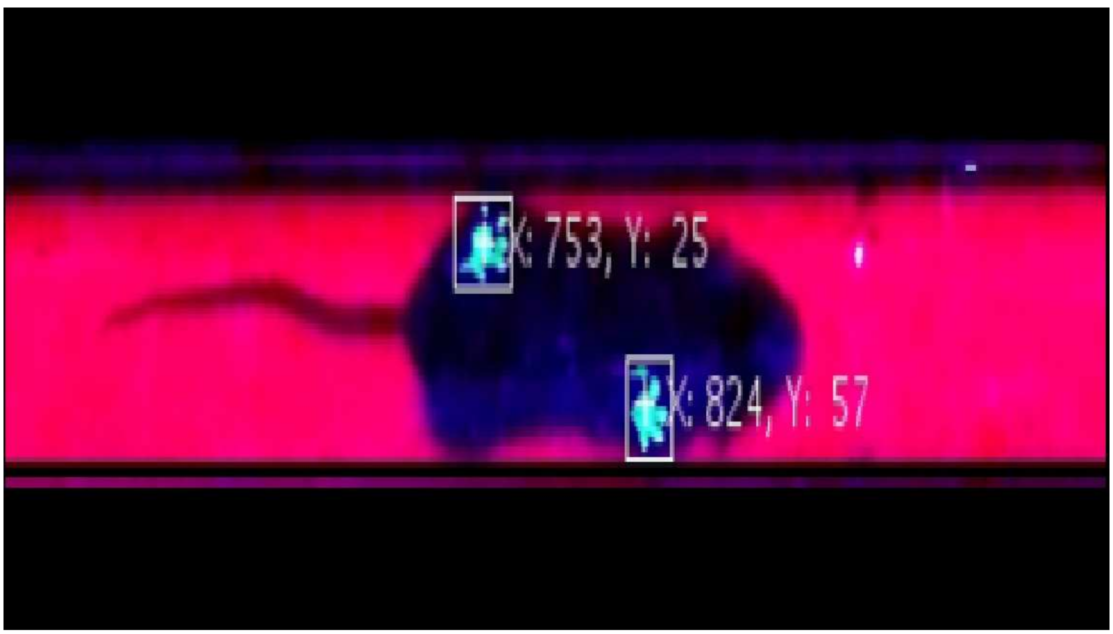

FIGURE 2.29. Zoomed-in trackway showing detected paws

2.4.3. **Paw Classification**.

After detecting all the locations of paws in the video, the next task is classification of the paws. There are minimum one and maximum four paws present in each frame, and these paws need to be matched with their same corresponding paws in previous and successive frames. Also, there may be mouse's tail or nose detected in some frames or some random noise may be present. This redundant data needs to be eliminated. Main difficulty with paw classification is the rear paws usually acquire the same locations as the front paws are lifted. So, most of the locations co-ordinates are repeated if you look at the data as a whole. Paw classification is important for calculating all gait metrics.

After acquiring all co-ordinates of paws in all frames, we divide the frames by the number of co-ordinates detected in each frame. We start by analyzing all frames with four objects detected. These are usually the frames with the mouse standing on all of its four paws with rare exception of tail or nose detected. Detected four paws are fairly easy to classify into four categories of *Left-Front, Right-Front, Left-Rear, and Right-Rear.* The four detected objects are divided into these four categories based on their X and Y co-ordinates. The two objects with higher X co-ordinates are classified as the front paws and other two are classified as the rear paws. Then the front and rear paws are again divided into left and rear based on their Y co-ordinate. With the mouse running from left to right direction, the right paws are the ones with smaller Y co-ordinates.

After classifying all the frames with four objects we move on to frames with three detected objects. We divide the three objects into two categories of left and right based on Y co-ordinates. The one category with two objects are then subdivided into front and rear based on X co-ordinates. The third object is not yet divided further. Then we move onto frames with two detected objects. Here the objects are only divided into left and right categories based on their Y co-ordinates.

After this, we go in recursive fashion from the four classified objects to their previous and successive frames. With predefined distance threshold, any unclassified paw with a distance less than the predefined threshold is assigned as the same paw. We move in both forward and

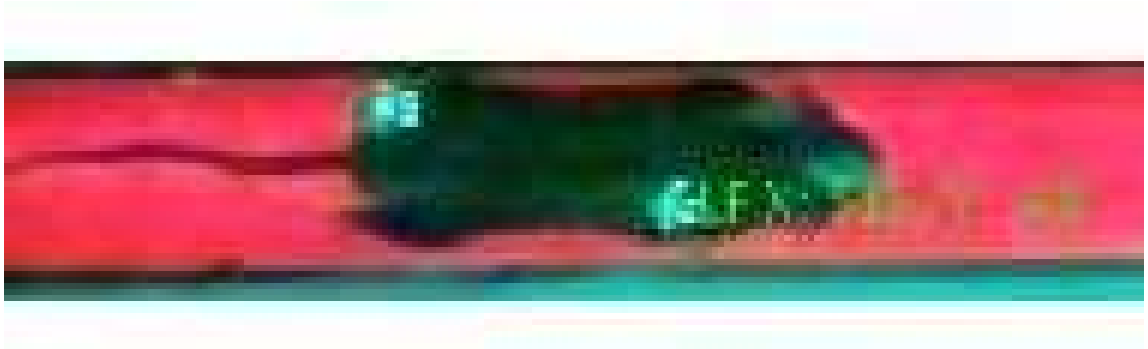

FIGURE 2.30. Zoomed-in trackway showing “Left Front (LF)” paw classified

backward direction for predefined number of frames. This predefined distance is finalized based on large number of tests.

2.4.4. **Gait Metrics**.

After the classification of paws different gait metrics were calculated. These gait metrics are useful in analyzing the the progress of disease or for tracking the recovery.

Along with these quantitative gait metrics some graphical representations of the gait analysis is also generated.

Paw intensity : Figure 2.31 and Figure 2.32 show a 3-D representation of paw pressure for a paw making contact with the glass floor. As explained before, images are recorded in RGB format where each of the pixel in image is assigned a color value for R, G, and B from 0 to 255. For calculating the paw pressure, all the values of green color are recorded around the detected paw co-ordinates. These values are then mapped into a 3-D image. The smoothing of the curve is achieved by interpolating the values using spline filter.

Table 2.1. Definitions of gait parameters

Parameter	Definition
Run duration	Time of finishing entire run in seconds
Stride length	The distance between two consecutive travels in the same paw
Swing duration	The duration of no contact of paw with glass in seconds
Swing speed	The ratio of stride length to swing duration
Stance	The time of paws in contact with the glass in seconds
Duty cycle	The percentage of stance over sum of stance and swing duration
Cadence	Steps per seconds in the trial
Base of support	The distance between fore limbs and hind limbs at maximum area
Diagonal dual support	
Three point support	
Four point support	The relative duration of simultaneous contact of limbs with the glass plate
Average area	Average area of paw contacting the glass plate
Average intensity	Average pressure of paw contacting the glass plate

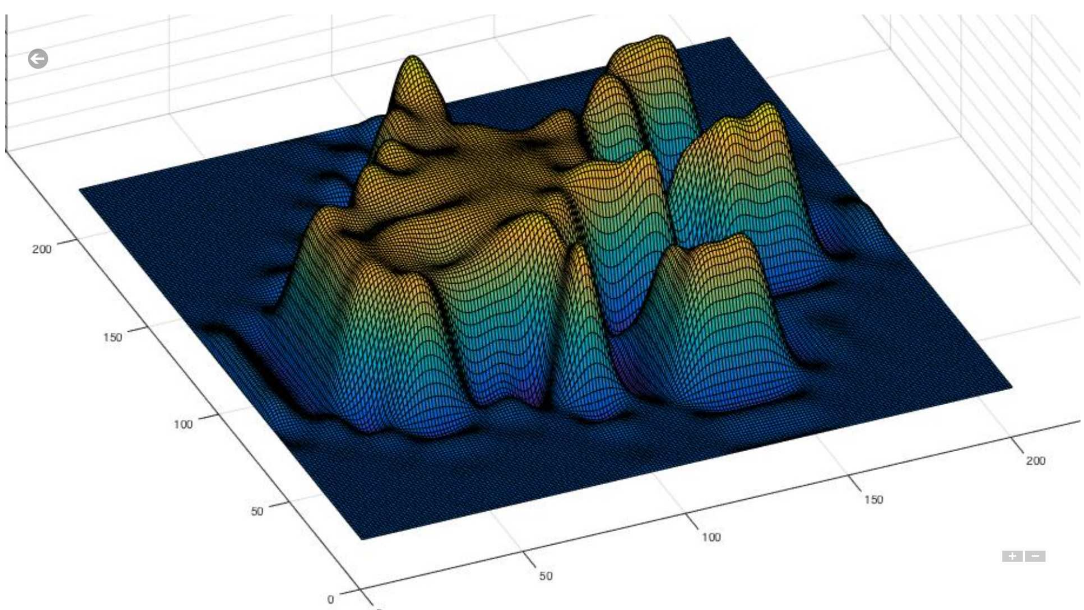

Figure 2.31. Paw intensity (side view)

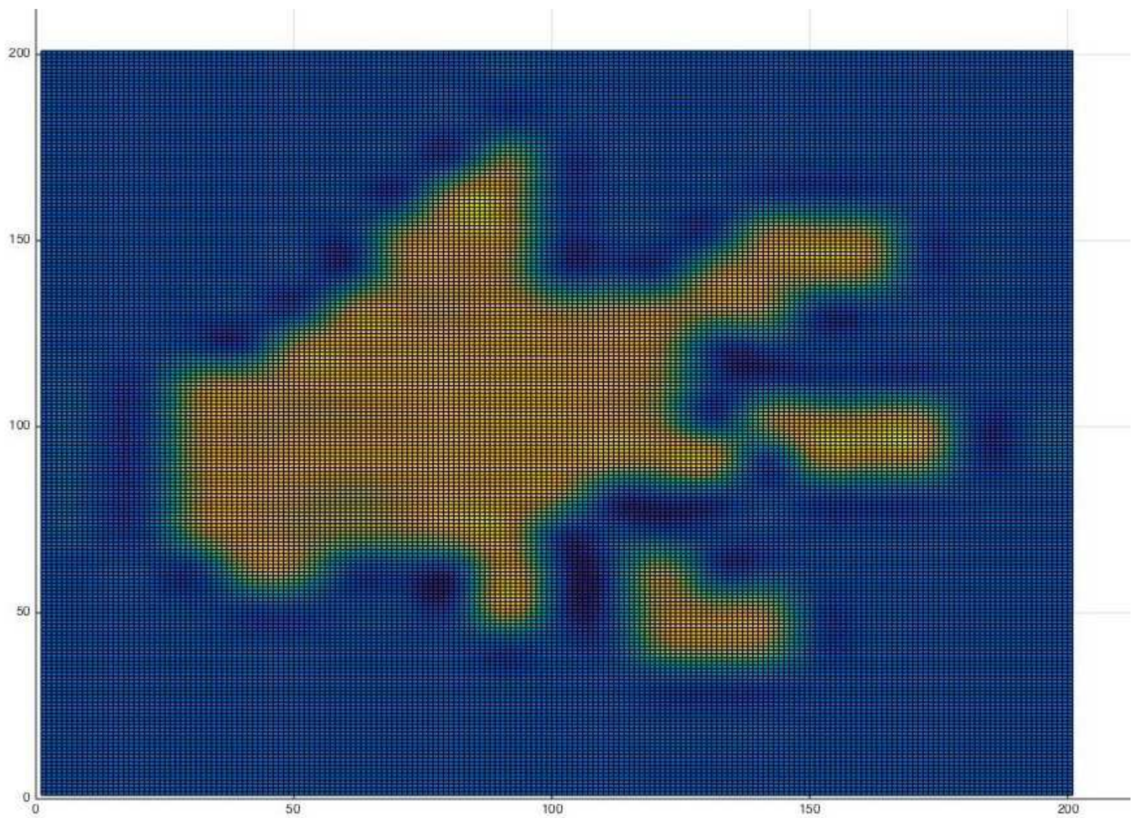

FIGURE 2.32. Paw intensity (top view)

The paw intensity images are useful in representing the exact area of paws where a mouse is putting pressure while walking. These images in a way represent weight distribution of the mouse while walking.

Composite Paws : Composite paw generates an image with all the detected paws on a single strip of glass walkway. This kind of representation is useful for analyzing the combination of footfalls or the paw support during a run. This is graphical way of showing which paws are making contact with the glass plate simultaneously. All the detected paws are classified first for this. Then for representation, all the green valued pixels around the centroids of the detected co-ordinates are first pseudo colored to differentiate between different paws, and then plotted in the image.

Distance vs Time : Figure 2.33 shows distance covered by each paw against time. This image is a combined graphical representation for run duration, stride length, swing duration, and stance. This representation is particularly useful for summarizing multiple runs in a single image.

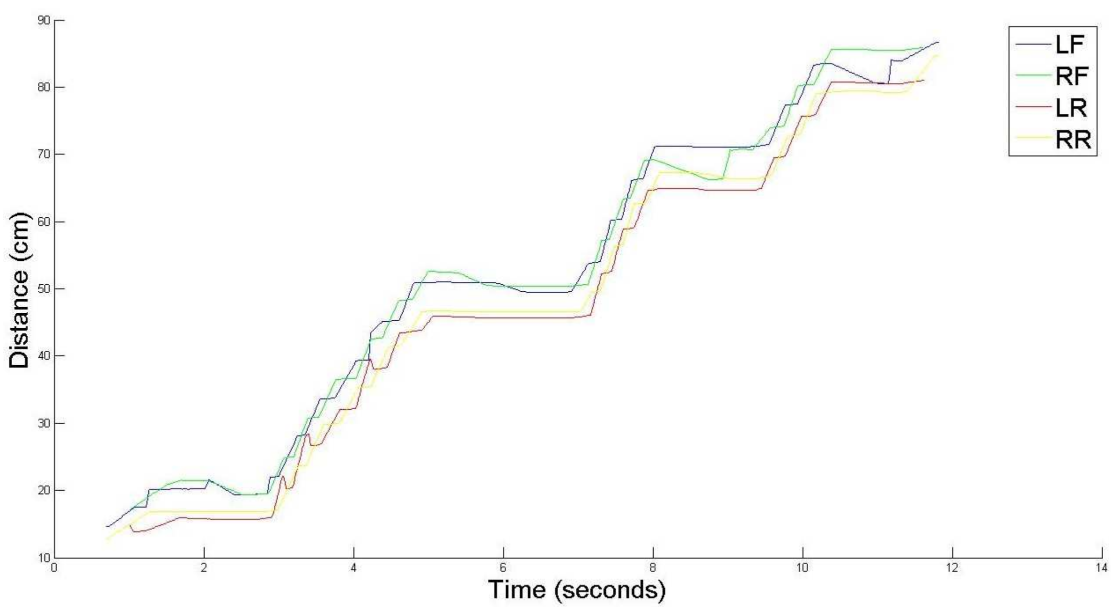

FIGURE 2.33. Distance vs Time graph for mouse run

2.4.5. Graphical User Interface.

After completion of coding for implementing image processing algorithms, a need for development of Graphical User Interface (GUI) arose. Although the code implemented was able to perform all the required tasks, still prior knowledge of MATLAB software was needed to for using the code. As the aim of developing gait analysis device was to make the device usable for everyone, it still came short on the user friendliness. For this reason developing a GUI was a necessity.

Requirements : The GUI must not require more than basic knowledge of using Windows or Mac. It should be able to analyze the video files. The generated gait metrics should be presented in easily understandable manner. There should be option for saving the analyzed data in easily portable file formats. The GUI should be able to generate graphical representation of the gait analysis. The GUI should be able to process multiple videos at a time.

Implementation : The GUI is developed using the GUIDE feature of MATLAB. The developed GUI is able to load and process multiple videos. Analyzed videos can be opened in the GUI itself or can be saved in '*.avi' format which can run on all the video processing softwares on Windows and Mac. Analyzed gait metrics are presented in tabular form in easily understandable manner. The metrics table can be ported to '*.csv' format to be used in other softwares such as MS Excel. Graphical analysis is generated in GUI itself and can be saved as '*.JPG' or '*.PNG' formats. All these task are performed using simple buttons created on GUI and do not require any prior knowledge of MATLAB to be used.

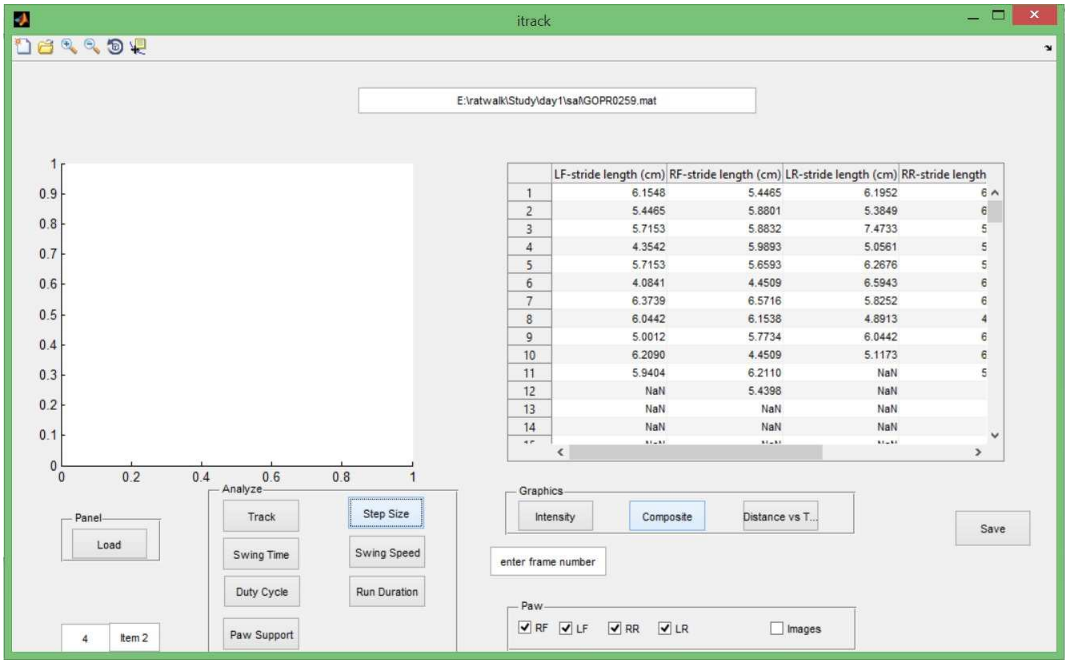

FIGURE 2.34. Graphical User Interface (GUI) window

CHAPTER 3

Results

3.1. Introduction

In this section, we will present the experimental results of the study conducted on live animals using the Mark III device. Data presented includes raw image data acquired by the device during experiment and data reported to user after analysis. A performance analysis of the system is also detailed.

3.2. Experimental Setup

The study started with three different groups of healthy mice. Total period of the study was 15 days. Each group contained 12 mice at the beginning of the study. First group was treated with *Saline* throughout the experiment. Second group was injected with *MPTP* on the days 1, 5, 9 and 13. The third group was treated with *MPTP* on the same days along with the oral gavage of *C-DIM12* compounds dissolved in corn oil (or corn oil vehicle control). On the 14th day mice were terminated. Behavior was assessed at these time points using the gait acquisition device. After the treatment each mouse was allowed to run on the glass trackway with optimally preset lighting. The trackway was wiped with alcohol solution after every run to clean any excretion left by mice during the run. Each mouse was allowed to run twice for the sake obtaining better test results. A run with fewer number of stops was selected for analysis of gait metrics. Acquired videos were analyzed on an independent computer with the image processing algorithms.

3.3. Mice Training

Testing has proven that it is extremely important to train mice to run across device before conducting any real experiments. This ensures that animal is comfortable with device and knows what is expected of it. It makes animal more cooperative and actual experiment becomes less time consuming. Food incentive method was used for training mice prior to experiment. Food was kept at the unloading end of the trackway while mouse was allowed to run on glass plate. This trains the animal to walk across trackway for reward. Also, mouse were given a slight push to enter the walkway after setting it on loading end of trackway. This trains it to leave house as soon as it enters one. This training was done for three days prior to the actual experiment. Such training methods can result in better gait data with minimizing idle time exploration of trackway during actual experiment runs.

3.4. Gait Analysis

Each video was analyzed using the image processing algorithms on an independent computer. MATLAB was used for implementing the image processing algorithms. Analysis performed for each video was presented in form of tables containing gait metrics. Most of the gait metrics (such as stride length, swing time, stance, paw intensity) are calculated for each individual paw and noted with Left Front (LF), Right Front (RF), Left Rear (LR), Right Rear (RR). Other gait metrics (such as run time, paw support) do not require classification into individual paws. The analyzed gait metrics are compared with their counterparts from different groups of mice on each day. Then each days progress is compared at the end to note the overall change.

3.4.1. Stride Length.

A change in stride length was observed as days progressed. Group with *MPTP* showed the most decrease in stride lengths compared to *Saline* and *MPTP + C-DIM12* groups. Stride lengths in latter two groups of mice were also decreased but the change was not as significant as in the *MPTP* group.

The pattern was consistent in all the four paws.

3.4.2. Run Duration.

Run duration was found to be increased as days progressed. *MPTP* group on last day showed the most change compared to its result on first day. While the other two groups also showed increase in run duration, still the amount of increase was less in these groups compared the *MPTP* group.

3.4.3. Paw Support.

Paw support was calculated in three different categories viz. Diagonal Dual Support, Three Point Support and Four Point Support. Each category indicates relative duration of simultaneous contact of limbs with the glass plate. As days progressed, there was dip in diagonal dual support and increase in three point and four point support in all three groups. The increase in four point support was more in all groups compared to the increase in three point support. The *MPTP* group showed slightly more change than the *Saline* and *MPTP + C-DIM12* groups in four point support.

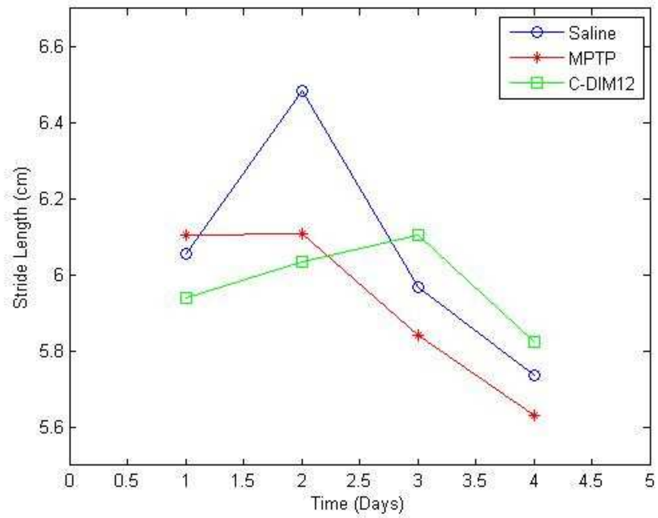

FIGURE 3.1. Changes in "Stride Length": Left Front Paw

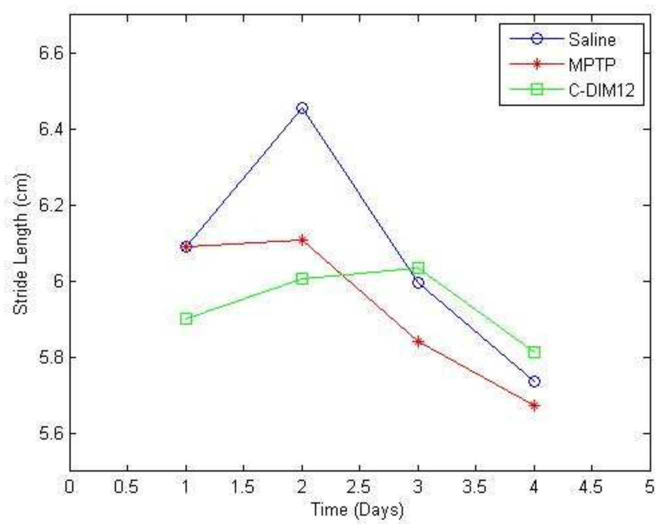

FIGURE 3.2. Changes in "Stride Length": Right Front Paw

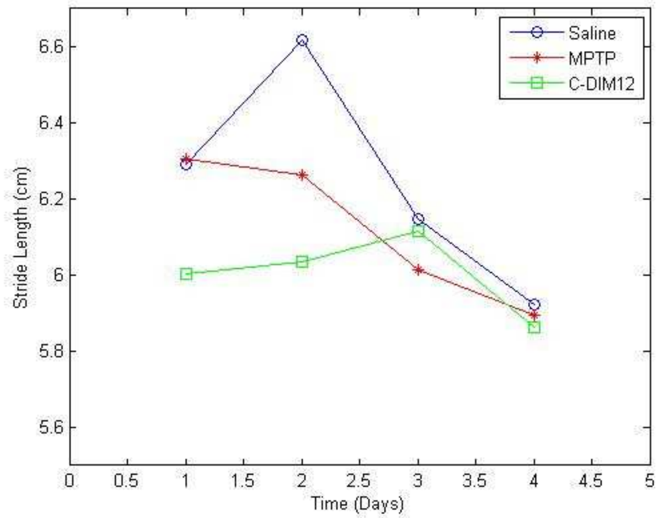

FIGURE 3.3. Changes in "Stride Length": Left Rear Paw

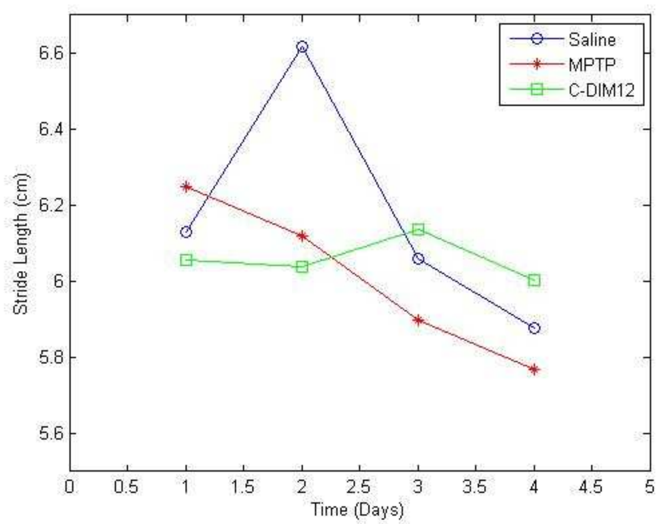

FIGURE 3.4. Changes in "Stride Length": Right Rear Paw

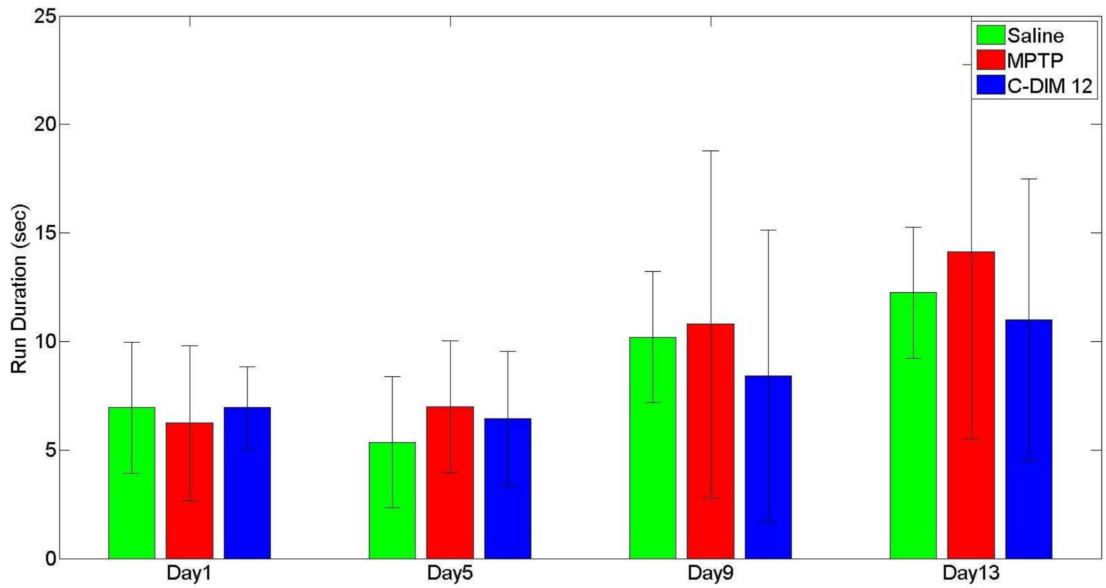

FIGURE 3.5. Changes in “Run Duration”

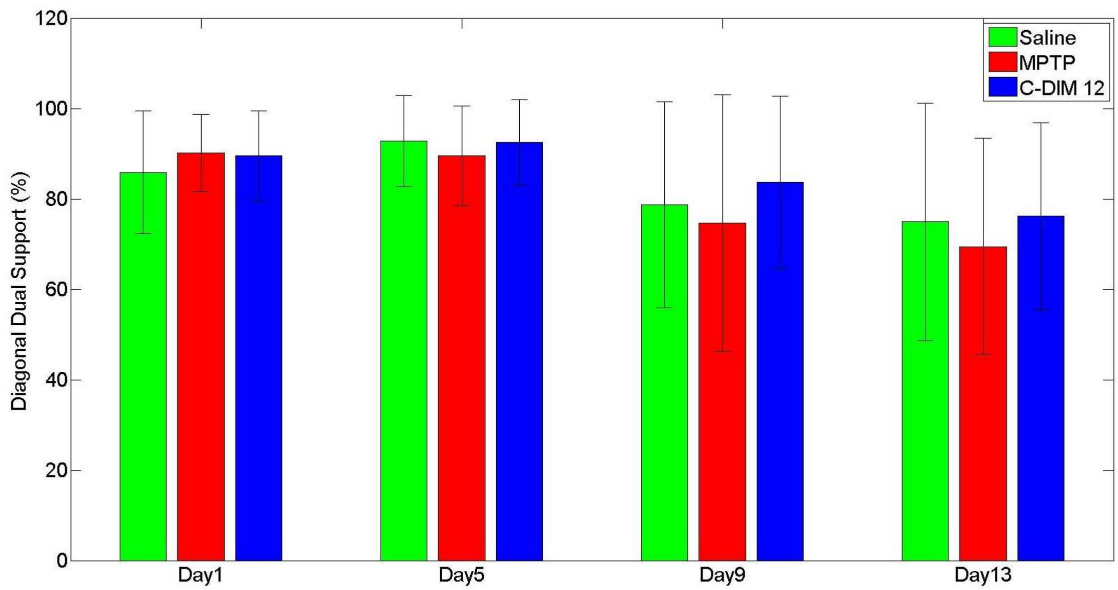

FIGURE 3.6. Changes in “Diagonal Dual Support”

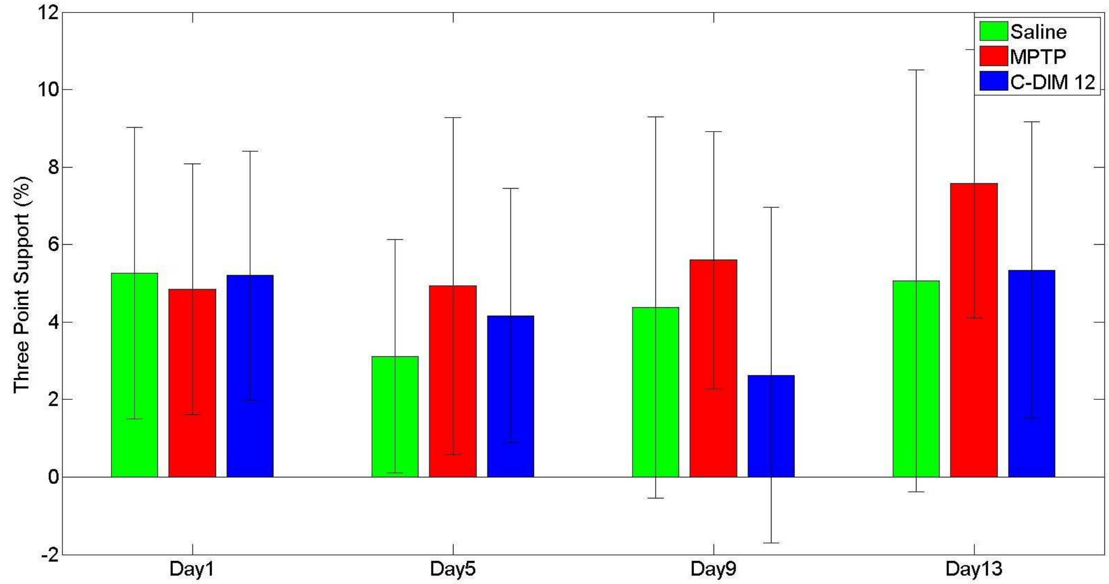

FIGURE 3.7. Changes in "Three Point Support"

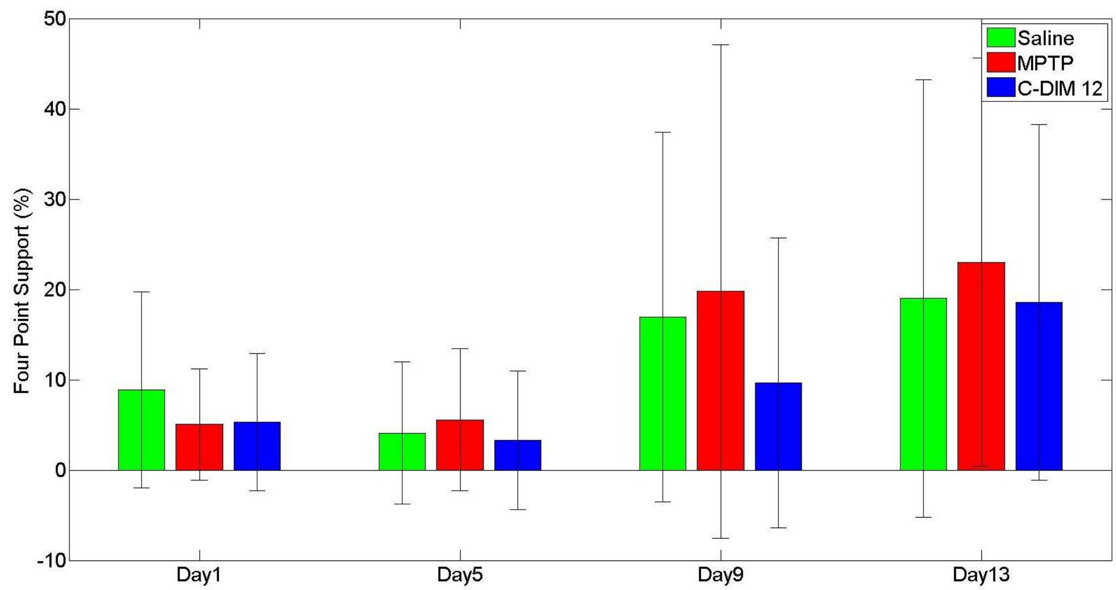

FIGURE 3.8. Changes in "Four Point Support"

3.4.4. Cadence.

There was significant decrease in cadence in all three groups as days progressed. The decrease in *MPTP* group was more prominent compared to the other two groups.

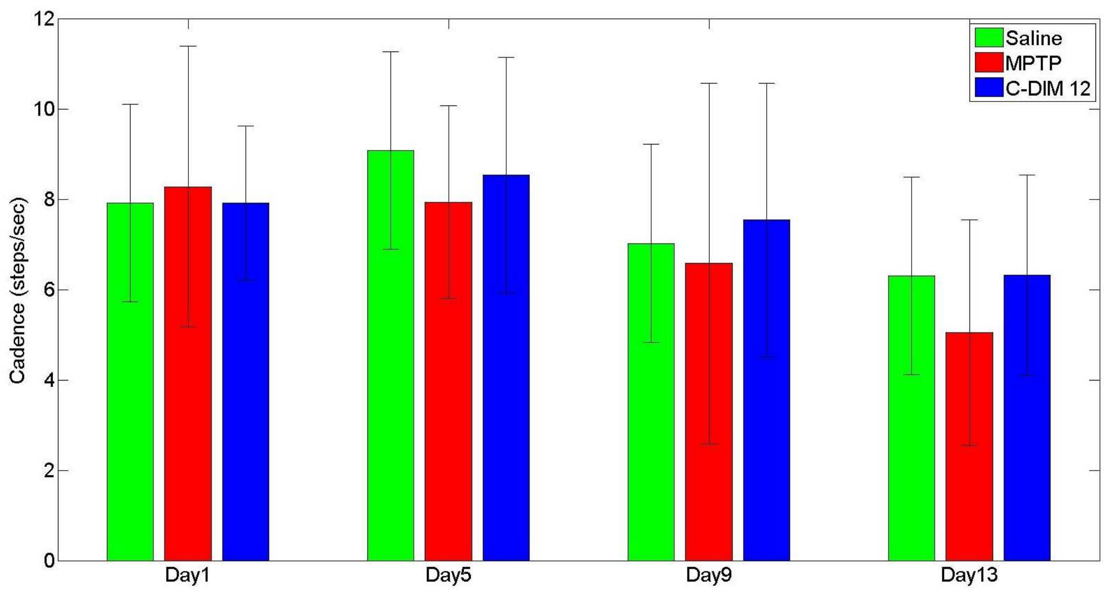

Figure 3.9. Changes in "Cadence"

3.4.5. Other Gait Metrics.

Other gait metrics such as stance, duty cycle, base of support did not show any notable changes throughout the experiment.

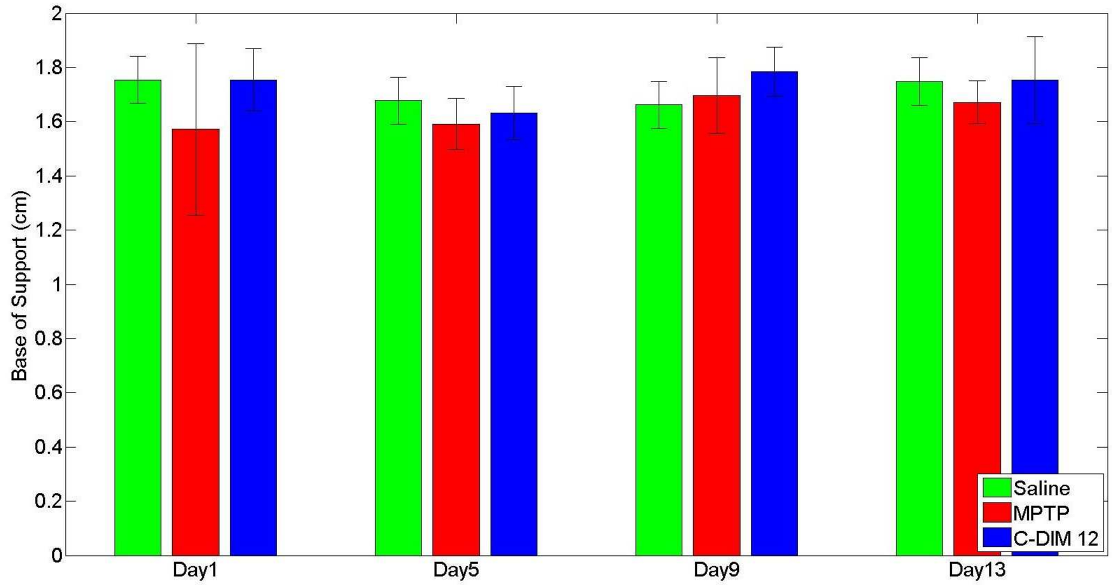

FIGURE 3.10. Changes in "Base of Support"

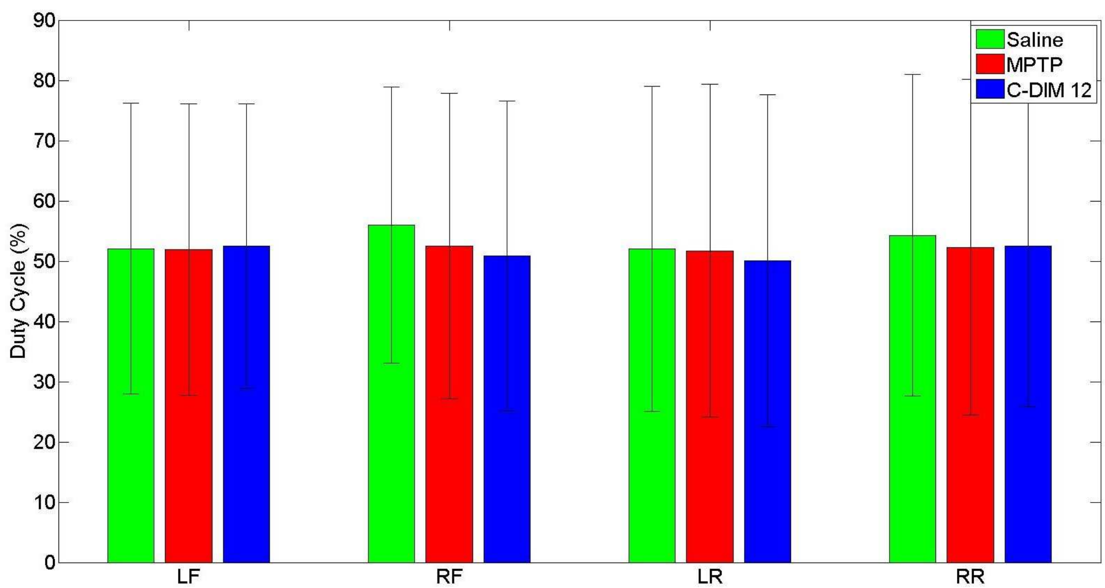

FIGURE 3.11. Changes in "Duty Cycle"

CHAPTER 4

Conclusion

4.1. Introduction

This chapter provides analysis of data provided by gait acquisition system along with analysis of the system. First, analyzed data generated by the system is presented. Next, explanation for using this data for tracking recovery is given. Then, possible ideas for implementing machine learning are suggested. Efficiency of the detection algorithm is discussed. This is followed by alternatives ways for improving the system in the future. The chapter is concluded with an overview of the entire project.

4.2. Gait Data

Raw gait data is presented in EXCEL sheet. This data presents X and Y coordinates for each paw for each frame of a video. X and Y coordinates reported are the pixel values in an image frame. Top left corner in the image represents base of axes. i.e. The values for X

	LF-stride length (cm)	RF-stride length (cm)	LR-stride length (cm)	RR-stride length (cm)	Diag Stride L2R (cm)
1	6.1548	5.4465	6.1952	6.8690	3.8673
2	5.4465	5.8801	5.3849	6.2110	3.8976
3	5.7153	5.8832	7.4733	5.4948	3.8465
4	4.3542	5.9893	5.0561	5.9351	3.8465
5	5.7153	5.6593	6.2676	5.3322	3.8976
6	4.0841	4.4509	6.5943	6.8690	3.9181
7	6.3739	6.5716	5.8252	6.3189	3.8673
8	6.0442	6.1538	4.8913	4.6167	3.8166
9	5.0012	5.7734	6.0442	6.3225	3.8166
10	6.2090	4.4509	5.1173	6.4307	3.8166
11	5.9404	6.2110	NaN	5.5538	3.8166

Figure 4.1. Raw gait data

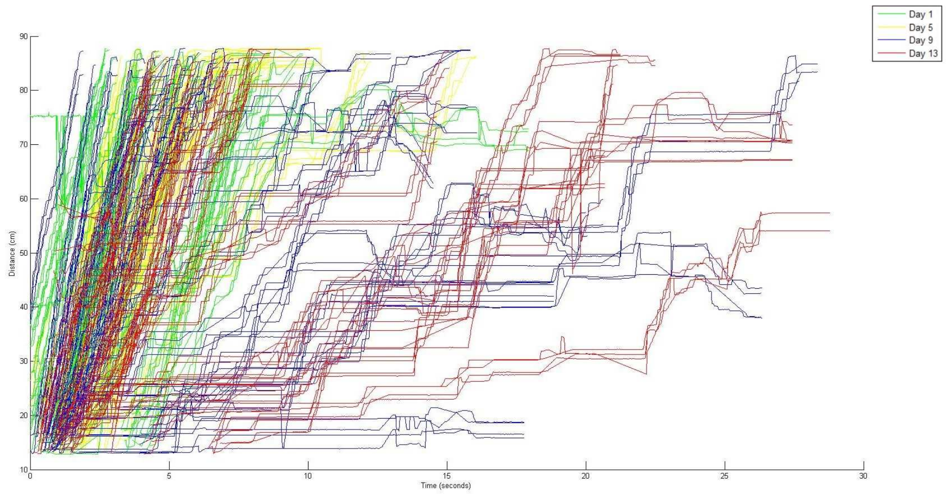

FIGURE 4.2. Distance vs Time: "MPTP group" all four days

coordinates increase as you move from left to right in the image. The values for Y coordinates increase as you from from top to bottom in the image.

4.3. Gait Data Analysis

Coordinates data generated for each paw is used to calculate useful gait metrics. The coordinates are used for calculating spatial gait metrics such as stride length and base of support. The frame numbers are used for calculating temporal data such as swing speed, stance and run duration. Comparing average values of these gait metrics between different groups of mice on different days indicate overall changes in the gait. Interlimb coordination can be observed by looking at the composite image generated for each run. From figure 4.2 we can see that a healthy mouse just runs across the trackway with interlimb coordination between the diagonal paws. While figure 4.3 shows that a sick mouse tends to stop a lot with more interlimb coordination between three paws and sometime all four paws.

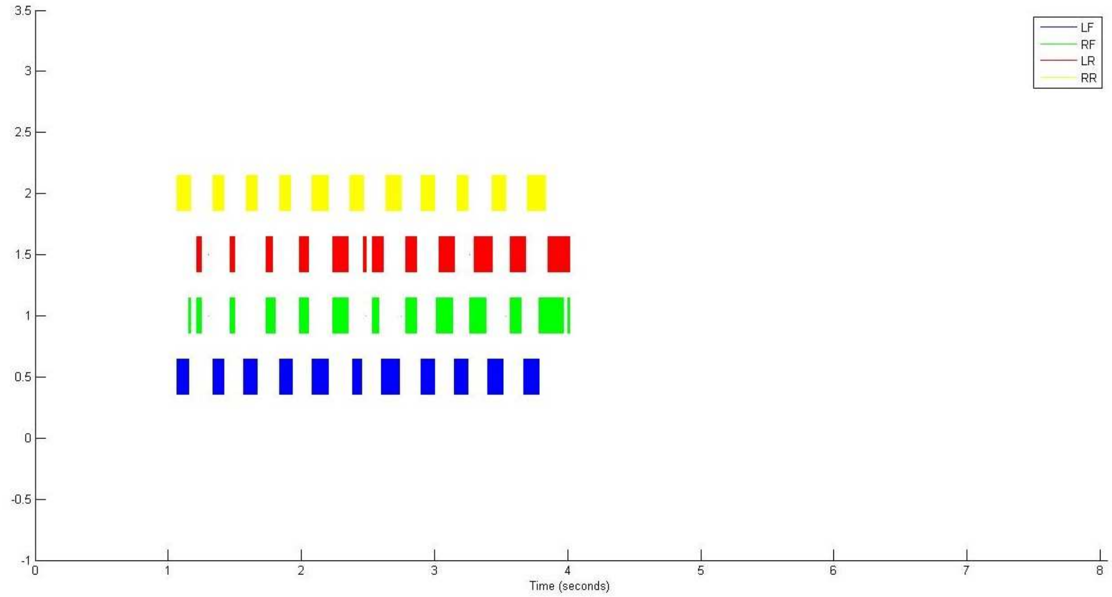

FIGURE 4.3. Interlimb Coordination: Healthy Mouse

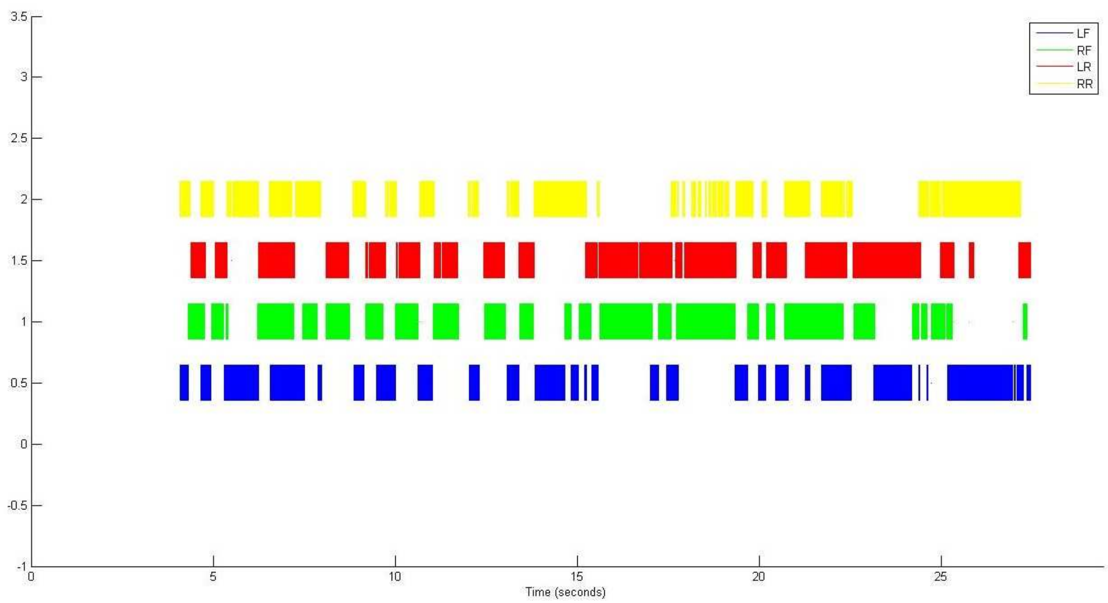

FIGURE 4.4. Interlimb Coordination: Sick Mouse

4.4. Tracking Gait Recovery

Gait metrics calculated are useful for tracking the recovery of injured mice. Behavior analysis plays major role in analyzing recovery during neural injuries. By observing the gait data, we can say if a particular mouse is making progress in the right direction or not. In particular, experimental study that we performed on live animals indicates positive changes in gait for mice group treated with *MPTP + C-DIM12* compared to mice group treated with *MPTP*. When we look at the changes in stride length as days progress in the study, we can see that the graph for *MPTP + C-DIM12* is in-between *Saline* and *MPTP*. This indicates that change in stride length for mice treated with *MPTP + C-DIM12* was in positive direction and we can say that it showed progress towards recovery. These changes in stride lengths are in accordance with the known theory.

Figure 4.2 shows combined graphs of "distance vs time" for "MPTP" mice group in all four experimentation days. We can see that, green lines representing first day are clustered towards left side of the graph. This shows the mice were crossing the track very quickly without much in-between stopping on the first day. As days are progressed, the graph tends to move towards right hand side. The red lines representing last day of the experiment are mostly clustered towards right hand side indicating slow moving mice with many in-between stops. This graphical representation confirms the initial assumption of PD slowing the movement of mice.

4.5. Machine Learning

The last image of combined "distance vs time" graphs beacon need for machine learning techniques to automatically classify healthy, sick, and recovering mice. We tried to implement hierarchical clustering to apply classify mice into different groups. For applying this, longest common sub-sequence (LCSS) was calculated for each run. If two runs have common coordinates then their LCSS score would be high and vice-verse. The LCSS is scored in the range from 0 to 1. If two runs have same coordinates then their LCSS would be scored 1 and two runs with no common sub-sequence would have LCSS 0. Then the runs are clustered in agglomerative way to form dendrograms. We observe that four major clusters are formed in the result indicating four days of data.

4.6. Hit/Miss/False Detection Ratio

Hit/Miss/False (H:M:F) detection ratio is the percentage of positive paw identification (hits), to missed paw identification (misses), to false identification (false detects). A positive paw identification denotes correct detection and classification of paws present in each frame. A missed detection is failing to detect paw present in any frame. A false detection is wrongly identifying an object as paw present in the frame or miss-classification of the detected paw. Goal of the image processing algorithm is to generate 100% positive detection.

A missed detection can be caused by low contrast. This way the area where paw makes contact with glass plate is not illuminated enough to be detected through the paw detection threshold. Or it can be caused when a paw is being lifted and area of contact is so small that it does not get detected through paw clustering threshold. A missed detection causes gap in acquired gait data and can result in compromising gait metrics.

A false detection can be caused by some random noise or object outside paw large enough to be falsely detected as a paw. Most of the times it is caused by either mouse's tail or nose making contact with the glass plate resulting in illuminated area. False detection can also mean miss-classification of paws. It usually happens when a mouse is walking way past center line of the glass plate causing the algorithm to miss-classify detected paws with opposite side (left and right) paws. Also, a false detection is possible when one of the paw is just being lifted, making algorithm miss-classify the detected paws with opposite end (front and rear) paws.

Average score for H:M:F was found to be 92.1: 2.3: 5.6. But, for some particular cases where a mouse was not walking straight ahead and kept turning in the track, the H:M:F ratio was 84.3: 0.9: 14.8. Compared to this, the H:M:F ration for mouse completing near perfect run with minimum stops was found to be 96.0: 1.4: 2.6. The false detection ratio was higher in turning mice because of miss-classification between left and right paws. These missed and false detections can be manually corrected by editing the correct coordinates into the excel spreadsheets. This way wrong calculation in gait metrics can be avoided.

4.7. System Refinement

Although the system met with all the basic requirements for the device, it can still be improved in future. Refinement can thought in two different aspects, hardware and software of the system.

Hardware : An easily improvable thing about the system is replacing the existing camera with an even better camera that can provide higher frame rates and resolution. Presently, cameras are available in market which can record videos even at 4k (3840x2160) resolution with 120 frames per second. But these cameras are really expensive and their use is not feasible with the goal to build a device in affordable price. But as the technology is improving every day, there is possibility of prices for such cameras dropping in future. This will enable use such cameras which can improve gait acquisition.

Another thing can be improved is the trackway. The glass plate can be mechanically tilted to create upward and downwards slopes for experimenting mice gait for varied conditions. This can be achieved with adding mechanical adjustments to the stand to tilt the glass from one end. This would create complication for captured videos, as the distance from the camera would be different for different ends creating optical illusions.

Other improvements regarding the lighting, enclosure, and accommodation space are user specific and should be adjustable for experimenting different sized rodents.

Software : The detection algorithm is ever improving task to achieve that ultimate goal of H:M:F ratio of 100:0:0. With better resolution of camera, the algorithm can be modified to achieve better results. Descriptors for recognizing paw shapes can be implemented to avoid false detection of tail or nose. Machine learning techniques can be implemented in more successful manner if more raw data is available to correctly train the neural network. The graphical user interface can be improved in future as per the end user requirements.

Only increase in regular use of the system for gait analysis can lead the way for creating better system.

4.8. Final Review

The final device captures gait data with help of GoPro HERO 3+ camera. Videos are analyzed on independent computer using MATLAB software. Analyzed videos can detect paws present in the video frames and can classify them into left front, right front, left rear, and right rear categories. Coordinates for detected paws are used for calculating various gait metrics useful for analysis. The device made to capture gait data is robust and easily transportable. The device can be improved, by changing individual parts such as camera, without any need for building whole device again. Image processing algorithms implemented for detection and classification of paws are reliable, and present the calculated gait metrics in easily understandable formats.

The device fails short on capturing videos at the highest possible resolution and frame rates available in market. The paw detection and classification algorithms is susceptible to missed and false detections. Several aspects of useful gait information, such as paw rotation, are still not implemented. Computation takes significant time to be performed and is not done at real time. Therefore all analysis needs to be performed post experiment.

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Pseudocodes for Image Processing Algorithms:

Green Pixel Threshold

for pixel number is less than width of image **do**

    for pixel number is less than height of image **do**

        if pixel's green value is greater than threshold **then**

            Mark pixel as green-pixel

        end if

    end for

end for

Proximity Clustering

if distance(green-pixel$_1$, green-pixel$_2$) is less than threshold **then**

    Rename green-pixel$_2$ as green-pixel$_1$

end if

Cluster Sizing

if Cluster size is greater than threshold$_{small}$ **then**

    if Cluster size is less than threshold$_{large}$ **then**

        Mark cluster as detected-paw

        Return centroid for the detected-paw

    end if

end if

Four Paw Classification

if Number of detected-paw in frame equal to four **then**

Arrange detected-paws in increasing order according to X co-rdinates

Arrange first two and last two detected-paws in increasing order according to Y co-ordinates

Classify first detected-paw as Left Rear

Classify first detected-paw as Right Rear

Classify first detected-paw as Left Front

Classify first detected-paw as Right Front

end if

Unclassified Paw Identification

for Frame number is less than or greater than threshold **do**

if Distance (classified-paw, unclassified-paw) is less than threshold **then**

Rename unclassified-paw same as that of classified-paw

end if

end for