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Technical note

Low-cost three-dimensional gait analysis system for mice with an infrared depth sensor

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ABSTRACT

Three-dimensional (3D) open-field gait analysis of mice is an essential procedure in genetic and nerve regeneration research. Existing gait analysis systems are generally expensive and may interfere with the natural behaviors of mice because of optical markers and transparent floors. In contrast, the proposed system captures the subjects shape from beneath using a low-cost infrared depth sensor (Microsoft Kinect) and an opaque infrared pass filter. This means that we can track footprints and 3D paw-tip positions without optical markers or a transparent floor, thereby preventing any behavioral changes. Our experimental results suggest with healthy mice that they are more active on opaque floors and spend more time in the center of the open-field, when compared with transparent floors. The proposed system detected footprints with a comparable performance to existing systems, and precisely tracked the 3D paw-tip positions in the depth image coordinates.

1. Introduction

Rodent gait analysis in an open-field is an essential procedure in genetic and nerve regeneration research (Leroy et al., 2009a; Sheets et al., 2013). For example, the first stage of standard mice phenotyping protocols (SHIRPA (Gailus-Durner et al., 2005) and modified-SHIRPA (Masuya et al., 2005)) include a qualitative scaling of the subject's gait in an open-field. Another example is the widely used rating scales (Basso, Beattie, Bresnahan Locomotor Rating Scale (BBB) (Basso et al., 1995) and the Basso Mouse Scale (BMS) (Basso et al., 2006)) used for regeneration research into spinal cord injury, which focus on the subject's hind limb gait in an open field. These protocols depend on human observations and manipulations. They are, therefore, subjective and difficult to replicate precisely. To solve this problem, we need an automated analysis system that is objective and repeatable. Several researchers have proposed automated analysis systems for open-field tests. However, most of these systems focus on the subject's locomotor activity (Zurn et al., 2005; Tort et al., 2006) or behavior classification (Noldus et al., 2001; Giancardo et al., 2013), and do not directly measure limb movements. Foot tracking systems (Vlamings et al., 2007; Crone et al., 2009) and marker-based motion capture systems (Courtine et al., 2008; Oota et al., 2009) have generally been used to measure limb movements. However, these systems affect the subject, and are therefore unsuitable for observing naturalistic behavior. In existing footprint tracking systems, the subject is placed on a transparent so that their footprints can be measured from underneath. The transparent floor, however, may induce discomfort in the subject and promote acrophobic behaviors (Van Abeelen and Kroes, 1967; Owen et al., 1970). Marker-based motion capture systems may also cause changes in a subject's behavior, for example, they may attempt to remove the attached markers. For these reasons, there is currently no automated gait analysis system that does not induce acrophobia or other behavioral changes. Moreover, most existing footprint-tracking or marker-based motion capture systems are expensive, which is a barrier to gait analysis systems because many researchers have insufficient

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funding (Dell et al., 2014). The aim of this study was to develop a low-cost gait analysis system that can measure the subject's gait in an open-field apparatus without any effects on its behavior.

Our approach to gait analysis is to observe the subject from underneath using an infrared depth sensor. Because the limbs of mice usually move underneath their bodies, this bottom-view setup can observe richer locomotive information than top-view or side-view setups. This idea was inspired by previous studies that captured subjects in an open-field from underneath a transparent floor (Leroy et al., 2009a,b). In contrast to those studies, the floor of our system is covered with infrared-pass filters so that the subjects behave naturally without being influenced by acrophobia. To observe the subject through the infrared-pass filters, we used a Kinect^{TM} (Microsoft, Redmond, WA) infrared depth sensor. Although, Kinect was originally developed as a human motion sensor (Han et al., 2013), it is sufficiently accurate to measure a three-dimensional (3D) mouse gait. Using the Kinect's depth map, our system can track the subject's footprint and paw-tip movements without any marker equipment. To track the 3D movements of body parts, we used a modified Dijkstra's algorithm called the AGEX (Accumulative Geodesic EXtrema) algorithm (Plagemann et al., 2010). The AGEX algorithm is a data driven algorithm that can robustly detect 3D extrema such as noses, tails and paw-tips without requiring a body model (Baak et al., 2013). Furthermore, a Kinect is cheaper than the sensors used in existing gait system (e.g., high-speed or multiple infrared cameras).

In this study, we conducted two experiments with healthy mice. We first experiment investigated the behavioral differences of the subjects in transparent and opaque (infrared-pass filters installed) conditions. The second experiment evaluated the tracking errors of the two-dimensional (2D) positions of the footprints and the 3D position of the paws.

1.1. Related works

A number of systems for measuring rodents' gait and posture changes are currently available. A detailed history of the automated observation of rodents was reviewed in Noldus et al. (2001), Ou-Yang et al. (2011), and Dell et al. (2014), so we only list notable research and systems that are closely related to this study (Table 1).

Position tracking is the traditional method of assessing the level of activity of a subject (Zurn et al., 2005; Publicover et al., 2009). Recently, Kinects have been used to track the positions of subjects in open-field settings (Ou-Yang et al., 2011). There are two benefits to using a Kinect for position tracking: (1) it is robust against surrounding light conditions, and (2) it can capture subjects in dark conditions, which is important because mice are more active in darker environments (Valentinuzzi et al., 2000). These advantages mean that a behavioral analysis system that uses a Kinect can be used in all light conditions.

2D body part (head, center of body and tail) tracking systems (Noldus et al., 2001; Clark et al., 2007; Lo et al., 2008) can determine the subject's direction. These systems can estimate the subject's interest in objects or other subjects, and validate the subject's social function.

Footprint tracking systems (Vlamings et al., 2007; Crone et al., 2009; Okamoto et al., 2011) are used to evaluate the subject's locomotive functionality. Footprint frequencies and positions depend on the condition of the subject's joints or muscles, which can be affected by arthritis (Ueno and Yamashita, 2011; Vrinten and Hamers, 2003) or nerve injuries (Vlamings et al., 2007; Neumann et al., 2009). Some systems use a treadmill (Crone et al., 2009) or wheel (Okamoto et al., 2011) to capture images of the subject in a static position, and result in high-resolution images that can distinguish the subject's toes. CatWalk^{TM} measures the pressure map produced by the paws on the ground using an optics-based pressure sensor that detects distortions in the plate. This sensor collects more gait information than other footprint tracking systems and allows the subject to move freely. This system has been shown to be very sensitive and objective when assessing motor impairments in rodents (Vlamings et al., 2007).

For studies that require more detailed information than footprints, marker-present motion capture systems such as the KinemaTracer (Ito, 2008) are used to track the 3D positions of the paws. MotoRater (Zörner et al., 2010) is another marker-present motion capture system, which can measure simple walking, wading, swimming, beam walking, and skilled ladder walking. The subject's body parts are marked with colors, and simultaneously tracked from three sides (bottom, left and right). The subject is placed in a rectangular pathway that is a beam, a ladder, a pool, or a path with a transparent floor to its home cage.

To provide possible improvements over the above systems, we developed a low-cost tracking method for 3D positions of rodent paws and 2D footprint positions in an open-field environment, with no more effect on the subject's behavior than the open-field test. Our proposed system allows subjects to behave more naturally in the open field using marker-less tracking and infrared measurements. Marker-less tracking avoids marker equipment or coloring, which can change the subject's behavior. The floor of our system was covered with infrared pass filters, which prevented the subject's acrophobia.

1.2. Paper organization

The rest of the paper is organized as follows. In Section 2, we explain our method (including the hardware setup and software implementation) and the experimental procedures and evaluation process. Section 3 contains the results of our experiments where we evaluated the accuracy of our system by comparing the result with ground-truth data marked by human mouse-tracking operators. In Section 4, we discuss the properties of our system, possible improvements, and limitations. Section 5 concludes this study.

2. Materials and methods

2.1. Animals

Thirteen 8-week-old male C57BL/6J mice (CLEA Japan, Inc., Tokyo, Japan) were used in our experiments. Eight were used for the first experiment to assess the effect of the floor opacity, and the other five were used in the second experiment to evaluate the accuracy of our tracking algorithm. None of the subjects had previously been used in the experiments. The subjects were housed under a 12-h light–dark cycle (lights on at 8:00) with controlled humidity and temperature. Food and water were available ad libitum. The animals were allowed to adapt to the experimental room for at least 16 h before the experiment. All experiments were performed during the light phase of the cycle (10:00–16:00).

All experimental procedures were approved by the local ethics committee established in the Nara Institute of Science and Technology.

2.2. Hardware and software environment

The system is composed of an open-field apparatus, a Kinect sensor, and a personal computer (Fig. 1). The open field is a square of 400 mm $\times$ 400 mm and the height of the surrounding wall is 320 mm. The Kinect device is fixed 430 mm below the floor so that the entire open-field area can be captured by the device. For the experiment in the opaque conditions, the floor of the open field was covered with tiled infrared-pass filters (FUJIFILM IR-80 (Fuji Film, Tokyo, Japan)), which are commonly used in commercial cameras.