

## DigiGait Imaging and Data Collection

The paradigm for **DigiGait** imaging is ventral view capture of the underside of the subjects' paws. By analogy, imagine you are running on an exercise treadmill, but that the treadmill belt and platform are transparent, with a camera looking up at the underside of your shoes. The camera takes a rapid sequence of photos of the underside of your shoes to report back to you the posture and kinematics of your legs. With your animals, the camera is imaging each of the 4 paws as they advance towards, make contact with, and retreat from the moving treadmill belt. This is the essence of **DigiGait**, for which a US Patent was awarded for its unique solution to the study of gait analysis in animals, including humans.

With this understanding of the paradigm, it should be clear that image quality is important. Lighting, camera settings, and focus enhance the images of the paws, the raw data for analysis. The **DigiGait** analyses software subtracts out colors from the images that do not correspond to the color of the paws, which are most often red in color. The user ought to strive for natural looking images that give the best natural reflection of the red color of the paws against the background. The background is the remainder of the animal's body and the "white" backdrop afforded by the LED lamp above the animal. Usually this premise and optimal contrast is easily achieved in black and white animals. In brown colored animals, the application of red food coloring can enhance the discrimination between animal paws and background. Gently pick up the animal by the scruff, and use a sponge brush to apply **DigiGait** Imaging Contrast to the underside of the animal's paws. Be careful not to get any on the animal's body.

Overground walking speeds for mice are ~14-40 cm/s. On the treadmill, the lower speeds introduce a lot of variability into the walking patterns for healthy mice, and the higher speeds can be challenging for compromised animals, and even healthy mice, depending on the age and strain. Generally, setting the treadmill belt speed to 24 cm/s is a good place to start for most studies in mice. B6 mice generally top out at ~45 cm/s, and CD1 mice can exceed 80 cm/s. Mice execute ~3 strides/sec, but at the higher running speeds, can execute ~9 strides per second! Aim, then for ~5 seconds of imaging, to capture ~+16 strides for data analysis.

Key advantages to the **DigiGait** paradigm in comparison to the overground paradigm are a) the range of speeds one can explore; b) the ability to collect data based on numerous strides; c) having all animals in a study group walk the same speed or speeds. Differences in walking speed have a huge impact on gait and posture, rendering most of the data coming from the overground paradigm somewhat moot. As a training exercise, a researcher can take 2 or 3 mice and collect video at two walking speeds, say 18 cm/s and 24 cm/s. The researcher will see the distinct quantitative changes occurring in the same mice simply because of the increase or decrease in walking speeds. Imagine the frustration with having animals walk their own voluntary speeds across a lighted pathway, or across paper with ink on their paws, some traversing a fast speed, others traversing somewhat hesitantly, some not at all, and others deciding half way to stop and turn around and go back! All of these behaviors impose a great deal of variability into the gait metrics. Since even, for example, ~10% reductions in

stride length can be phenotypic of, for example, Parkinson's disease, these kinds of differences in stride length can simply come about from differences in walking speed.

**DigiGait** solves this very important confounder by empowering the researcher to have all of the animals walk the same speed or speeds. After placing the animal into the walking compartment, make sure the adjustable partitions/bumpers are positioned so that the entire mouse is in view of the camera. For naïve mice, a quick on/off of the treadmill belt is usually instruction enough for the animal to react to the moving belt and entrain itself to the treadmill walking. Tapping the bumper behind the animal into its hind quarters, and sometime just the audible noise of rattling the bumper/rod assembly within the walking compartment housing, encourages the animal to keep up with the moving belt. The primary purpose of the bumpers is to prevent toes and tails from coming into harm's way.

Make sure the **DigiGait** image capture software is capturing, just in case, for example, that the animal's motor phenotype is severe and it can only perform the test shortly and/or once. Once the animal is walking, aim to capture ~5 seconds of movie before terminating the treadmill and camera. Turning off the camera capture in advance of terminating the treadmill has advantages in the curating the movie, but of course turning the treadmill off as soon as possible has advantages for the animals' safety.

In the playback view, the user identifies an initial frame and an end frame depicting the animal performing the test. The edited movie may end up being ~1.5 seconds in length in the case of a severe dysfunctional phenotype, and as such the investigator may only be provided data based on ~4 or 5 strides, yet data that the investigator can use. However, usually the strategy described above yields data based on ~15 strides or so. Again, the data is based on the speed of the treadmill belt [which correlate to stride frequency of the animal] and the length of the movie.

Numerous strides from groups of animals at known and equivalent speeds will increase your repeatability, reduces your standard errors, and provide you much more meaningful data.

Thank you for using **DigiGait – better data from every mouse!**