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Musculoskeletal Soft Tissue Laboratory Spring 2018 Kivitz Progress Report

Griffin Kivitz
Washington University in St. Louis

Spencer Lake
Washington University in St. Louis

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Griffin Kivitz

Progress Report

Spring 2018

Musculoskeletal Soft Tissue Laboratory
This is a semester progress report for the spring 2018 semester working in the Musculoskeletal Soft Tissue Laboratory under the direction of Dr. Spencer Lake. I had two primary tasks/projects I was working on this semester. In chronological order, I was helping Alex Reiter with the editing of gait analysis images to rectify the noise and any problems with the initial code that may have had an impact on the filtering process. After that, I began designing an in vivo range of motion mechanical testing device for unilaterally injured rodents.

This report will be broken up by projects, and each project will have a brief overview to give context to my semester’s work.

**Gait Analysis:**

**Overview:**
One of the methods used to assess rodent function after a unilateral elbow LCL transection and anterior capsulotomy is gait analysis. Just like in humans, after injury, rodents will walk with a limp, and through gait analysis, we can determine quantitative parameters to characterize that limp. Some of these key parameters are symmetry of gait, time spend on each limb, and the distance between steps.

This gait analysis is performed using a high speed camera to take spatial-temporal measurements of a rodent walking through an arena. The arena is shown in fig. 1 below.

**Figure 1**

![Fig 1: The gait analysis arena has a mirror at 45 degrees positioned underneath the walking platform. This mirror allows the camera to record a side view and bottom view simultaneously.](image-url)
Utilizing the 45-degree mirror underneath the transparent walkway, the camera records the rat from the side view and from the bottom view. The side view shows the body of the rat and the time at which each limb touches the ground. The bottom view is used to find the location at which each limb touches the ground. After taking these recordings, a MATLAB code is used to filter the background from the side view, and filter everything but the hand/foot prints from the bottom view. The centroid of the rat and of each footprint is tracked with time. With these measurements, we can evaluate the function of the injured limb by determining the aforementioned parameters of gait symmetry, time spend on each limb, and distance of steps, among other parameters. These parameters are all normalized to velocity and weight to allow for comparisons between rats. The camera records at a frame rate of 500 fps to ensure that the proper foot strike and toe up times are recorded.

My Work:
With respect to gait analysis, my work consisted of manually editing images produced by the MATLAB code from the imported videos. A typical image can be found in fig. 2 below that shows the image before editing and the image after editing.

Figure 2

![Figure 2](image)

Fig. 2: The left image shows the image produced by MATLAB before editing. Note the random white specs (red box), the white stripes that are not entirely in the image (green box), and the two stripes that are joined by just a few pixels (blue box). After editing, these faults are removed, and the resulting image is shown on the right.
Editing these images includes removing any foot prints that are not completely in the image, removing any noise (specs) that are not footprints but appear as white pixels, splitting any footprints that may connect to one another within the image, and filling any negative space within a footprint.

With 9 rats, 10 trials per rat per week, and 6 weeks of gait measurements, this editing process had to be completed for roughly 540 images. In addition to this large number of images being time consuming, after completing the editing for about half of the images, we realized that the data itself was not being edited. There is an aspect of the code that generates numerical data based on the images we edit. There was a bug in the code that resulted in the edited images being saved, but the numerical data was not updated with the new edited images. Because this numerical data is used to determine numerical gait function, it was necessary to debug the code, and re-edit the images to make sure the numerical data was edited as well. I helped Alex Reiter complete the editing process.

**In Vivo Range of Motion Mechanical Testing:**

*Overview:*

Another important tool for evaluating function is range of motion mechanical testing. Currently, rodent limbs are mechanical loaded after sacrifice to determine the range of motion in the injured and contralateral limbs for a set maximum force. This range of motion testing is an indication of if the drug and physical therapy treatments were successful in preventing/breaking down the buildup of scar tissue, which would decrease range of motion. Figure 3 below shows the current range of motion testing after sacrifice.

**Figure 3**

![Figure 3: After sacrifice, the limbs are skinned and mechanically tested to assess range of motion. The left image shows the limb at full extension and the right image shows the limb at full flexion.](image-url)
**My Work:**
While range of motion testing after sacrifice is useful, it does not give any indication of how the range of motion of the rodent limbs change throughout their therapy. I sought to design an in vivo range of motion mechanical testing device to assess range of motion of the animals during their 6 weeks of therapy treatment. These measurements would allow for the evaluation of how the therapy helps range of motion with time. We could answer questions like: “Does the majority of range of motion return after 3 weeks? Is there any difference in range of motion between week 5 and week 6?”

This task proved much more challenging than I initially expected. The first aspect that was difficult was determining a way in which to fix the rat radius and ulna to the piece that will actually be moving. This is difficult because of all the loose skin and fat on the rat. The skin would stay fixed relative to the splint it was attached to, but the bone and muscle underneath the skin was not restricted at all. After trying rubber-bands and clamps, it was determined that the best way to fix the forearm to the splint was with the same elastic bandage used to immobilize the injured arm after surgery. This wrap allows for the force to be distributed over a large surface area of the arm, and it can be pulled tight enough to fix the arm in place.

The second, and more difficult challenge to overcome, was finding a way to fix the humerus in place. The humerus must be fixed because the range of motion is defined as the angle between the humerus and the radius-ulna complex. If the humerus is moved during loading, then the reference point for the angle to be measured is no longer constant. Fixing the humerus is a difficult task for the same reason as previously mentioned, there is too much skin and fat around that area. In fact, the majority of the humerus is under the skin and fat from the body of the rat and doesn’t extend very far out of that fat. Also, the humerus cannot be clamped or pinched because any external force on the triceps or biceps will restrict the range of motion. This is about where my work on this project ended for the semester.

**Future Work:**
Moving forward, we think the best way to fix the humerus is to manually hold in place by pinching the shoulder blade. This does not put any external force on the triceps or biceps, and also keeps the humerus in place. The forearm will be manually loaded through the range of motion with a torque sensor giving real time torque measurements. When the user sees that the torque measurements read the specified load, the user will stop loading the limb, and record the range of motion measured with a goniometer. We will only be evaluating extension because the splint, which is attached to the limb, will interfere with flexion testing. Also, because the limb is held in flexion during the immobilization period, extension is a more important measurement anyway. While this testing protocol is not nearly as controlled as that of the mechanical testing after sacrifice, it will still provide useful measurements. Also, this manual loading of the limb is similar to what is done in clinical practices.