**Fatigue Parameter Estimation Protocol**

The following is the procedure that should be followed to collect the necessary data to perform the muscle fatigue parameter estimation of the neuroprosthesis leg extension model. If at any time the participant asks to discontinue the tests STOP IMMEDIATELY. The participant may also discontinue any of the procedures by using the emergency stop button. Note that the fatigue parameter estimation protocol must come after the other parameter estimation protocol has been completed and parameters have been estimated for the participant. Do not run this procedure on the same day as the other parameter estimation protocol. Read the document in its entirety before conducting tests.

1. Test the emergency stop button:
   1. Plug the wireless receiver into the 9V battery.
   2. Build and run the Simulink model on the desktop titled SafetyTest.slx.
   3. When the model is running press the wireless button, which should stop terminate the Simulink model.
   4. If the model does not terminate DO NOT PROCEED WITH EXPERIMENTS. Try to resolve the problem and repeat the test of the emergency stop button. Do not proceed until this test has confirmed that the emergency stop button is working correctly.
2. Test the stimulator:
   1. Make sure that the stimulator unit is connected to the PC that you are running the experiments on through the RS232 port.
   2. Turn the stimulator on.
   3. Once the stimulator is on press the HV button to turn off the output of the stimulator.
   4. Connect one of the channels of the stimulator to a 100Ω resistor in parallel with an oscilloscope.
   5. Open the TestStim.slx Simulink model, set the channel to the output that the resistor is connected to, and set the amplitude to 20mA.
   6. Press the HV button on the stimulator, then build and run the TestStim.slx Simulink model.
   7. Use the oscilloscope to confirm that the stimulator parameters are the same as the ones that were specified in the settings of the stimulator block.
   8. Once you have completed the test stop the Simulink model and press the HV button again to turn off the output of the stimulator.
3. Place electrodes on quadriceps of the participant following directions from <http://www.axelgaard.com/>. Once the electrodes are place on the muscles and the wires are connected press the HV button to turn on the output stimulator.
4. Situate the participant in the leg extension machine:
   1. Make sure that the arm of the leg extension machine is unpinned (not locked in position).
   2. Have the participant sit in the leg extension machine, and adjust the back seat for comfort and to approximately align their knee joint with the axis of rotation of the leg extension machine.
   3. Strap their leg to the arm of the leg extension machine and adjust the position and orientation of the padded load cell for comfort.
   4. Pin the leg extension arm in an isometric configuration with the leg positioned as close to its natural equilibrium as possible.
   5. Plug in the power supply that powers the load cell of the leg extension machine.
5. Run parameter estimation tests:
   1. Open Main\_Fatigue.m.
   2. DO NOT RUN THE SCRIPT! Instead evaluate the script one section at a time, in order from top to bottom. This will allow you to evaluate results of each tests in between each procedure, give the participant rest periods if required, and give you time to prepare for the next procedure. You will be prompted by Matlab at the beginning of each procedure to ensure that you are prepared to proceed.
   3. Run the section of Main\_Fatigue.m titled “Preamble”. This will setup some things for the upcoming sections.
   4. Run the section of Main\_Fatigue.m titled “Subject Information”. This section will allow you to select the subject ID for the participant, and the leg that you are performing the procedure on.
   5. Run the section of Main\_Fatigue.m titled “Muscle Fatigue Test”. This will build and run a Simulink model that will potentiate the muscle with ten pulses, fatigue the muscle with two minutes of continuous stimulation, and finally measure the recovery of the muscle using ten short pulses of stimulation.
   6. Run the section of Main\_Fatigue.m titled “Save Collected Data”. This will create a .mat file of the results of the procedures. This file should be relocated to the secure location that has been designated by the investigators. Any previous versions or copies of the saved results should be deleted.