

INSTRUCTION MANUAL

DMC Software

Dynamic Muscle Control

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1.0 General Description

1. Overall Program execution is enabled by clicking on white arrow (Figure 1) top of window and stopped by clicking on the red button. Once the program is enabled, the user can switch between modules without turning the program off.

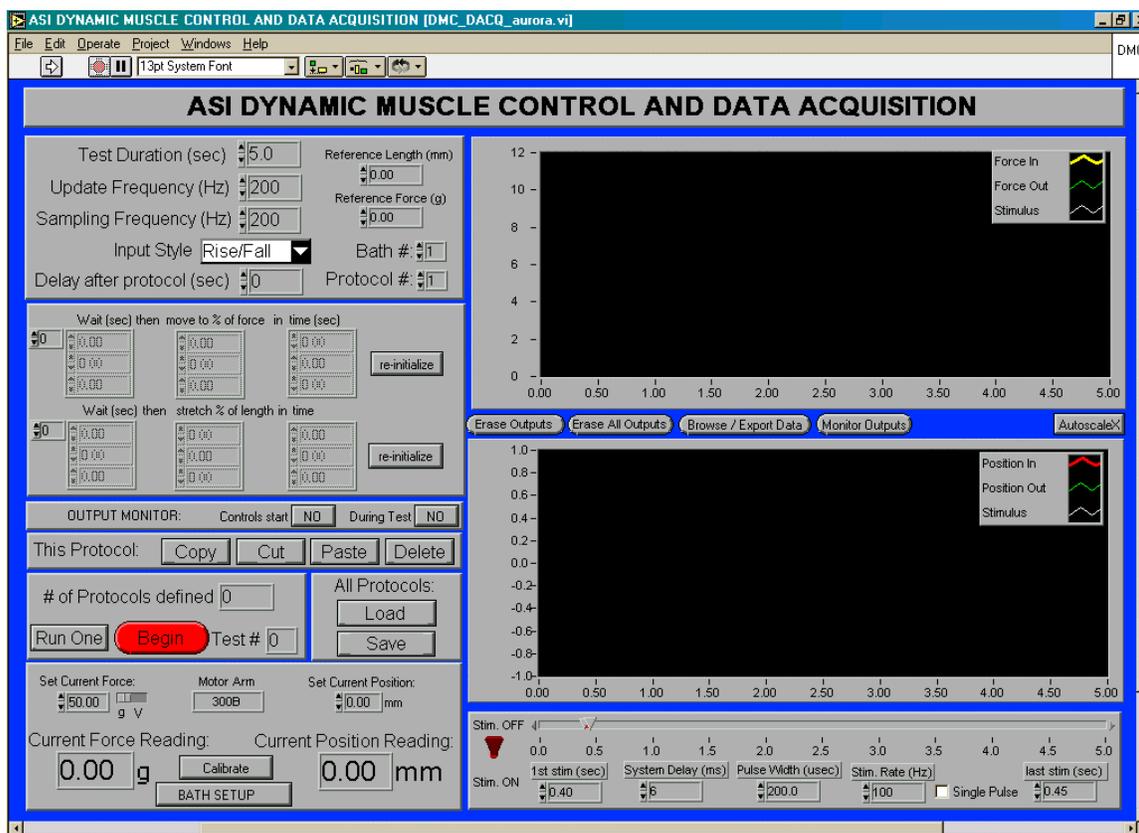


Figure 1. DMC control panel

2. The program has facility for,
 - a) Controlling any number of ASI, Inc. servomotor units. (Limited by D/A and A/D card capabilities only). The servomotors are designated baths with an associated number. Each bath corresponds to a specific configuration of D/A and A/D channels and devices, which can be set in the measurement setup sub-program to be discussed below. If multiple configurations have been defined, the desired bath or configuration is selected by left clicking the mouse on the arrow to the right of 'Bath #'.
 - b) Setting test duration from 1 second to several minutes (in sec). This duration is reflected in the data displays (i.e., 0-2 sec; 0-10 sec; etc.).
 - c) User selectable update frequency (1-10000 Hz): this is the frequency at which the data to control the servo arm force and position are streamed. If the arm is in a static position a low update frequency is adequate; if the arm is to move rapidly, a higher rate would be used. user selectable sampling frequency (1-

- 20000 Hz). Typically, a sampling frequency of 1000-2000 Hz is adequate for most muscle physiology applications. To observe phenomena of a given time scale select a sampling frequency of 10-20 times that equivalent frequency. For example, to observe muscle changes occurring on a time scale of 0.01 sec use a sampling frequency of $20 \times (1/0.01) = 2000$ Hz.
- d) However, to assess precise timing for the stimulation pulses, a frequency of 10000 Hz (for a stimulation pulse width of 0.2 ms) or greater is required. If this rate is not used, the stimulus will not be displayed.
 - e) Choice of 3 modules:
 - (1) **Rise/Fall**: Timing, rate and duration of changes in the position or force resistance of the arm as well as the timing, duration and frequency of stimulation can be programmed here. For example, a stretch protocol during an isometric contraction.
 - (2) **Muscle%**: For Force-velocity measures; stress-relaxation relations.
 - (3) **Sine Wave**: Sinusoidal position outputs.
 - f) Choice of two run modes:
 - (1) **Run One**: A single protocol is executed from the current baseline position
 - (2) **Run All**: All protocols defined in the series are run sequentially. Protocol series setup and manipulation is discussed below.

1.1 General Screen Description

1. The top left controls allow the user to set the test length and overall data acquisition parameters. The analog output rate (Update Frequency) must not equal the analog input rate (Sampling Frequency). The input style determines the type of waveform generation used to generate a protocol. The program always shows the **Rise/Fall** module upon startup. Other choices for the input style are 'Sine Wave' and 'Muscle Percent'. All input styles are discussed in detail below.
2. The 'bath #' control allows the user to select the bath that the current protocol should be performed on, in the case where multiple baths have been defined. More information on bath definition can be found in the section on measurement setup below. The 'protocol #' control allows the user to scroll through or add to the series of protocols active in the program. Any number of protocols can be defined and then executed in sequence. Manipulation of a protocol sequence and execution is discussed below in the section Protocol series manipulation.
3. The 'delay after protocol' control allows the user to specify the amount of rest time in between each protocol in a sequence. The value is ignored for the last protocol in a sequence and when the program is being used in 'Run One' mode. If the wait time remaining before the execution of the next protocol is greater than 5 seconds a monitor window will pop up showing the inputs in time currently observed from the selected bath. Tests can be aborted from this window

4. Introductory description of screen for **Rise/Fall** module: Inputs are provided so the user can set the duration before a change in force or position of the arm [wait (sec)]. Note: both force and position changes cannot currently be evoked simultaneously. The rate at which the arm should move [e.g., rise (mm/sec)]; and the duration over which the arm should move [time (sec)] can also be set. Settings to move the arm upward or change the arm load (maximum load depends on the model of servo motor) must be compensated by the same magnitude settings in the opposite direction to return the arm to its original starting position or force setting (or vice versa), if this is desired. Further notes on the controls available in the **Rise/Fall** module:
 - a) A series of inputs are possible to perform several position changes within a single protocol. The first three inputs are entered and then if a 4th or more are required, the user clicks on the arrows just to the left of and below the Wait column (currently reading zero) to enter the values.
 - b) The 'delete last row' button is used to clear the last row of inputs. New inputs can be entered or a different module such as the Muscle% or Sine Wave can be selected.
5. Below the inputs for the **Rise/Fall** module are the buttons for data monitoring and data acquisition start control. Clicking the first button controls whether or not a given protocol's execution start is controlled from the monitoring window. Clicking the second button controls whether or not the acquired data is displayed during the test.
6. Below the controls for data monitoring and protocol start are the protocol manipulation commands. The buttons allow individual protocols to be copied, moved or deleted. Protocol operations are discussed in more detail below in the section on protocol series manipulation.
7. The '# of protocols defined' indicator shows how many protocols are contained in the currently defined sequence. The 'Run One' or 'Run All' button is used to toggle between the 'Run One' and 'Run All' modes of the program. Clicking on the control toggles the mode. In 'Run One' mode, the program will execute only the currently displayed protocol when the 'Begin' button is pressed. In 'Run All' mode, the program will execute all defined protocols in sequence starting with protocol one. While the program is on, the 'Test #' displays the total number of collection runs + 1, and increments with each successive push of the 'Begin' button. **Note:** The value is reset to zero if the program is stopped and restarted or if all protocols are being run in sequence.
8. To the right of the 'Test #' indicator are the controls that allow a sequence of protocols to be reloaded (using 'Load') or saved (using 'Save'). Several example protocol series are included with the software package.
9. Below the 'Test #' indicator are the 'Set Current Force' and 'Set Current Position' controls. These controls allow the user to change the force resistance or position of the motor. The update from these controls to the motor is immediate in the 'Run One' mode and restricted in the 'Run All' mode. Details are given in the section Protocol Series manipulation. **Caution:** Since updates in 'Run One' mode are immediate, the arm

motion induced may cause sample damage. Also exercise caution when changing from 'Run All' mode back to 'Run One' mode.

10. The 'Current Force Reading' and 'Current Position Reading' show the current position of the arm and the amount of force on it. The current force reading can be used to fix the resting tension of a muscle prior to protocol execution.
11. The 'Calibrate' and 'Bath Setup' buttons bring up the calibration and measurement setup windows respectively. Each of these windows is discussed in separate sections below.
12. On the right of the front panel are two display windows, the upper for the force traces and the lower for the position traces. Between the graphs are the 'Erase Outputs', 'Erase All', 'Monitor Outputs', and 'Browse / Export Data' buttons. Pressing the 'Erase Outputs' button will remove the currently displayed output data. 'Erase All' removes all currently available output data. Press the 'Monitor Outputs' button to observe the outputs in a scrolling type, close to real time display, as shown below in Figure 10, except that pressing the continue button in this case will return the user to the front DMC panel. The monitor panel is useful for assessing whether or not steady state has been achieved in the bath or assessing the level of noise in the data. The 'Browse / Export Data' button is used to perform basic data analysis and export the collected data from the current protocol to an ASCII file. The ranges for the X and Y-axes can be user selected or auto scaled. The time base (X axis) is determined by the test duration setting noted above. The force (Y axis) can be set to change automatically with the range in the data collected. Right clicking with the mouse on the displays brings up a menu that includes Auto scale X or Auto scale Y-axes. If Auto scale is deselected then axes ranges can then be altered at the base (X) or left (Y) of the displays by clicking on the desired time point with the left button of the mouse and entering a new value. The lines representing 'Force Out, Position In and Position Out can be displayed in user selected colors and line widths. Select the line to be changed in the inset box for Force or Position using the mouse pointer and left click on the mouse. A window will appear with several options for adjusting the line parameters (e.g., line width), one of which is line color. The 'Stimulus' will only be displayed if the sampling rate is high enough, 10kHz or greater for a 200 μ s stimulation pulse width. The equivalent frequency for this pulse width is 5000 Hz (1/0.0002) and to observe the pulse the sampling rate must be at least double the maximum equivalent stimulus rate. The Stimulus line characteristics can also be changed. When the test length is changed the program automatically resets the x-axis range.
13. At the bottom right of the display is the **Stimulation Window** for setting the stimulation parameters. The user can use a slide bar (or digital inputs) to set the start and end of the stimulation, select the pulse width (200 μ s is typical for stimulation of mouse skeletal muscle; for muscle from rat or another mammalian source the pulse width should be determined by the user), and select the stimulation frequency (1-5000 Hz; 150-200 Hz would be suitable for most skeletal muscle applications). For single pulses, the user can select the 'Single Pulse' box, independent of the 'Stim Rate' setting. The system delay control is used to line up the output to the motor with the stimulation output. For the

system the software was tested on, a value of 6 ms gave accurate results. The value should be set using the **Sine Wave** module discussed below. Stimulation can be turned off with the switch at the left of the stimulation control box. This removes the stimulation controls from the display. Full control of stimulation depends on additional ASI hardware. Another option is triggering a TTL compatible stimulator from another vendor.

2.0 Input Styles

The DMC program provides three different input modules (styles): Rise/Fall, Muscle % and Sine Wave.

2.1 Rise/Fall Module

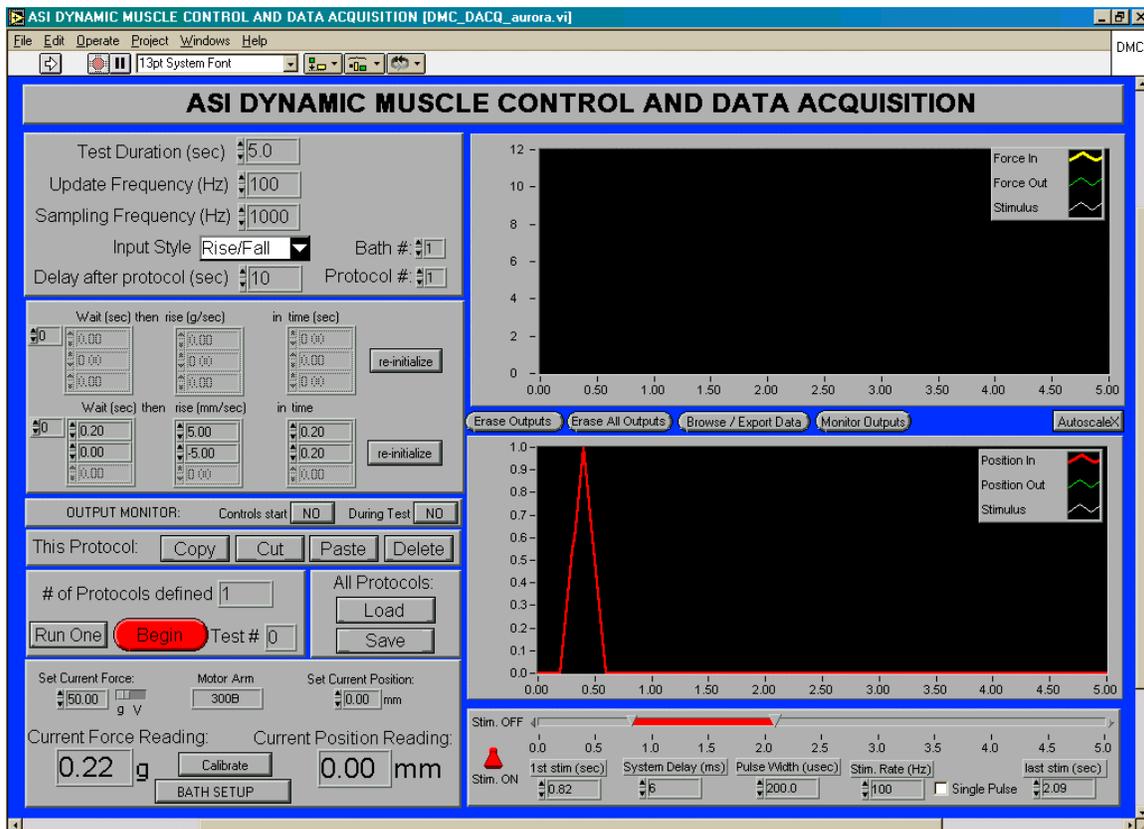


Figure 2. Rise / Fall module

The Rise/Fall module was discussed in some detail in section 1.1. An example is shown here in Figure 2. The input calls for a wait time of 0.7 sec followed by a 5 mm/sec ascent for a duration of 0.2 sec. The protocol continues in the second line with zero wait time followed by a 5 mm/sec descent for 0.2 sec again, returning the arm to the baseline position of 0 mm.

The input of force protocols follows that of the length protocols in the Rise/Fall module. Instead of a rate of change of position, a rate of change of force is entered.

2.2 Muscle % Module

The 'Muscle %' module is also very similar to the 'Rise/Fall' module and is shown in Figure 3.

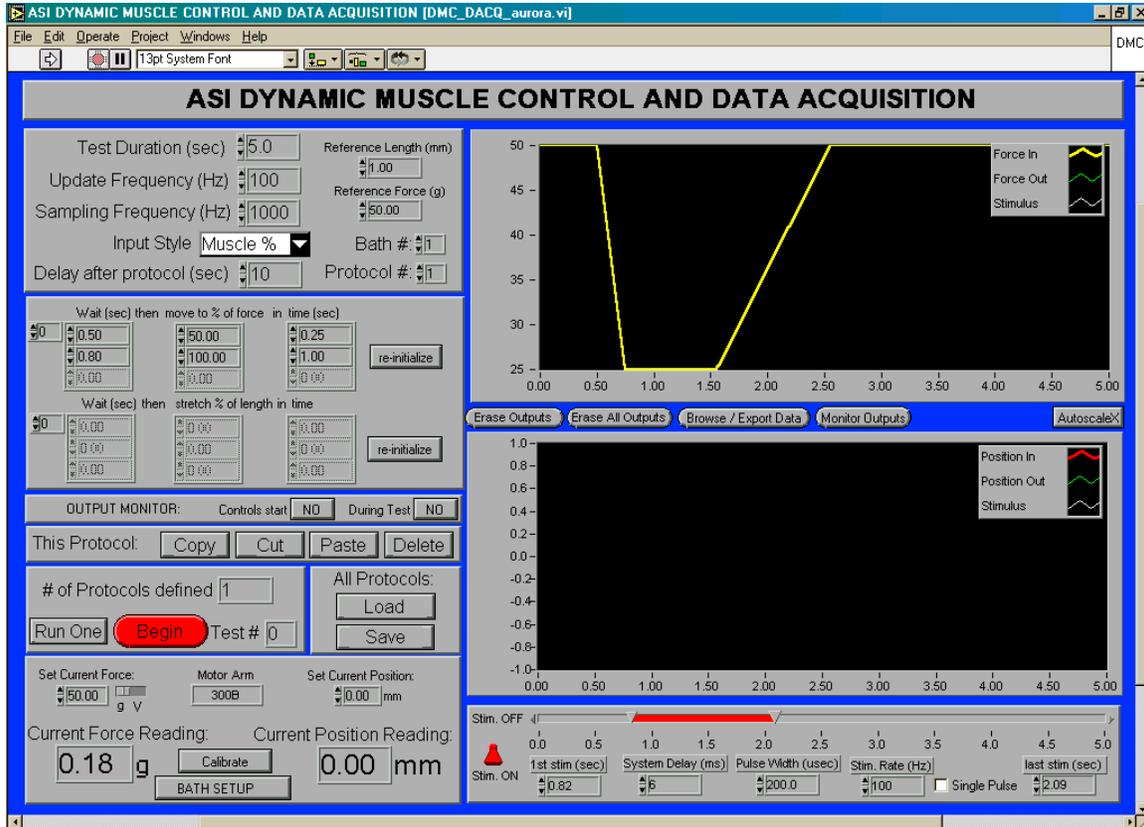


Figure 3. Muscle % module

Two additional inputs are provided for the 'Muscle %' module. The reference length and force must be entered in the controls above the bath number control. In the muscle percent module, all movements or changes in the arm resistance are made relative to these reference quantities. The main difference between the 'Rise / Fall' module and the 'Muscle %' module is that in the 'Muscle %' module, instead of specifying the rate of movement or change in arm resistance, the final position or resistance is specified using a percent of the appropriate reference value. In the example shown in Figure 3, the reference force is entered as 50 g. The protocol waits 0.5 sec then moves to 50 % of reference force in 0.25 sec. Then, the protocol waits 0.8 sec and returns the arm to 100 % of reference force in 1 sec. The graphical output shows the actual protocol to be executed. The advantage of this input style is that just changing the reference force will change the protocol completely. Length protocols are defined analogous to the force inputs with the reference quantity of course being the reference length specified.

2.3 Sine Wave Module

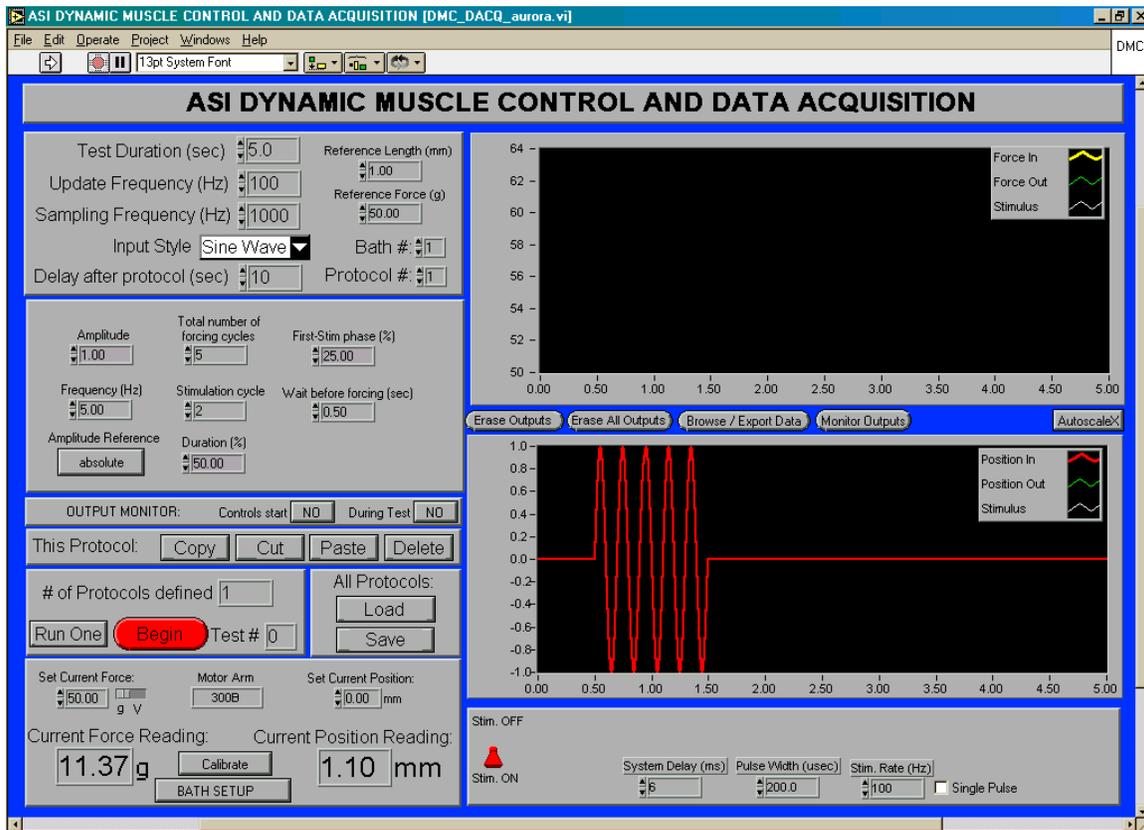


Figure 4. Sine wave module

Finally, the 'Sine Wave' module allows sinusoidal wave output in position. Figure 4 shows the setup for a wave train with 5 cycles at 5 Hz. The wait time prior to the wave train is specified as 0.5 seconds. The amplitude in this case is specified as 1 millimeter because the amplitude reference is set to absolute. Pressing the button will change the amplitude reference to 'percent length' where the reference length is again entered above the protocol # control. The stimulation parameters for the sine wave module are somewhat different. The module allows for stimulation to occur at a specific point in the wave train. In the example shown, stimulation is set to start on the second sine wave at 25% (90 degrees) of phase. The duration of the stimulation is specified as a percentage of the wave period. In this case, the stimulation will occur over 50% of the period, that is 0.1 sec. The number of pulses generated in this period is determined by the stimulation rate that can still be controlled from the bottom stimulation panel. Clicking on 'single pulse' will override the duration setting and issue only one pulse at the desired phase with respect to the sine wave. The system delay control is also determined using the sine wave module. The issue will be addressed later in this manual.

3.0 Protocol Series Manipulation

1. The DMC program allows the execution of any sequence of protocols employing any of the three modules (rise/fall, sine wave, muscle %). When the program is started, the program has one defined protocol. The protocol can be changed in all the ways discussed in this document. To add a protocol copy and then paste a selected protocol or click the up-arrow on the 'protocol #' control and a default protocol will be added after the currently displayed protocol. If you copy and paste the protocol is always pasted after the currently displayed protocol. Each protocol can be copied or cut or deleted, using the appropriate buttons to the left of the graphs. Each protocol has its own settings of test parameters including stimulation and acquisition parameters. The only parameters controlled from the DMC panel that cannot be independently controlled are the current position and force settings.
2. The current position and force settings are tied to the whole protocol sequence when the program is in the 'Run All' mode. Current position and force can be controlled for each protocol in the 'Run One' mode. The mode is changed by clicking on the button that displays the current mode (i.e. either 'Run One' or 'Run All'). Here, the position and force values are then immediately updated. The immediate update means **that rapid movement of the arm can occur** and these controls should be used with **extreme caution**. In the 'Run All' mode, the current position and force are those inherited from a previous protocol. For example, suppose that one wants to run a protocol that raises the arm to the 1mm position using the rise fall module. The protocol after this one should remember the position of the arm after the first protocol is run. The program remembers the current position and force of the arm through the set current force and length controls. The set current force and position controls can only be changed by running intermediate protocols that establish the new desired condition. The reason for handling protocol sequences in this way is that hardware limits can be kept track of more easily and that rapid movements of the arm can be prevented. To change the starting point (values of current force and position) in a sequence of protocols, change the current force and position inputs to the desired values in the 'Run One' mode and then enter the 'Run All' mode. Care should also be taken when switching from the 'Run All' mode back to the 'Run One' mode.
3. Each protocol series can be saved and reloaded using the 'Save' and 'Load' buttons. To copy a protocol from one saved series to another, it is possible to simply press the copy key for the desired protocol, load the protocol series requiring addition, and press the paste key at the protocol immediately preceding the desired location for the copied protocol.

4.0 Measurement Setup

Measurement setup is called up by pressing the 'Bath Setup' button in the main DMC window. The window is shown in Figure 5. The measurement setup of baths is not restricted to the physical existence of multiple baths. The bath definition can simply be used to keep track of different computer hardware or motor configurations. Each bath can be thought of as a test configuration and each test configuration is defined in this window in terms of A/D and D/A connections, hardware limits and conversion coefficients.

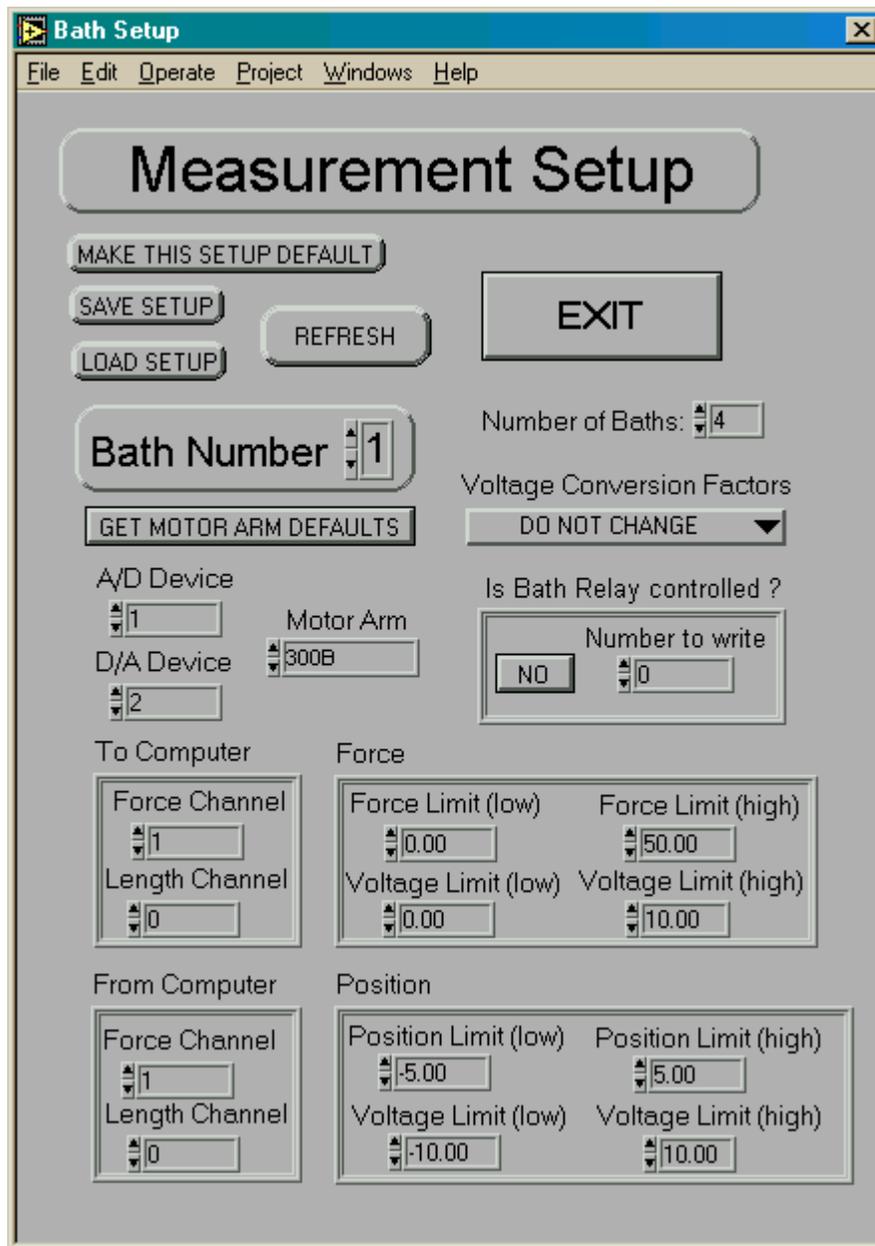


Figure 5. Measurement/Bath Setup Window

1. Pressing the 'Make this setup default' button will save the configuration as defaultconfig.ini. The defaultconfig.ini file is loaded every time you launch the DMC program.
2. Pressing the 'Save Setup' button will save the current setup. A file dialog window will pop up and the user will be able to enter a filename for the configuration to be saved.
3. Pressing the 'Load Setup' button will bring up a file dialog window to select a configuration file to be loaded.
4. Pressing the 'Refresh' button will reinitialize all the values on the screen and update the values according to for example newly loaded calibration coefficients.
5. Pressing the 'Exit' button will close the measurement setup window, implementing any changes that were made to the setup into the running of the program.
6. 'Number of Baths' indicates the total number of bath configurations you have or will define. To define two bath configurations when you currently only have one, increase this number.
7. 'One Bath' is shown as long as only one bath is defined (e.g. Number of Baths = 1). When 'Number of baths' is changed, the display will change and a bath number control will be available, titled 'Bath Number'. Choose which bath configuration you would like to check or update using this control.
8. Pressing the 'Get Motor Defaults' button will load the values of the selected standard ASI lever motor into the boxes labeled 'Force' and 'Position'. Alternatively, for other motors, the values can be entered by hand in the dialog boxes provided. The assumption for motor parameters is that force / position control of the motor occurs using the same calibration factor as force / position reading. Some older versions of motors have a ratio other than 1:1 and in this case the output data must be corrected manually.
9. The 'Voltage Conversion Factor' selector box has three choices for the calculation or retrieval of the conversion factors for force and position. The default choice is keeping the current conversion factors. To get the conversion factors from a previously saved calibration file, choose 'Get from calibration file'. To calculate the conversion factors from the linear limits entered into the 'Position' and 'Force' boxes, choose 'Recalculate from limits'. Pressing 'Refresh' now will initiate the calculation or bring up a file dialog window to load the desired calibration file. The conversion factors are obtained by assuming that the sensors are linear between the entered limits.
10. 'A/D Device' and 'D/A Device' are the device handle numbers of National Instruments boards for the respective hardware operation. For single card systems the device number is the same for A/D and D/A.

11. The 'Is the Bath Relay controlled' box should read 'No' for all standard systems. The digital number entered here is written to the first digital port of the card when this bath is selected. The control can be used for example to redirect the stimulation pulse into a certain bath. Such custom control hardware is available through ASI. More information is provided with those systems where the information here needs to be changed.
12. The 'To Computer' box should contain the appropriate channel numbers for force and length outputs from the lever motor.
13. The 'From Computer' box should contain the appropriate channel numbers for force and length inputs to the lever motor.
14. The 'Position' and 'Force' boxes have already been mentioned. For the low values enter corresponding values of position and voltage. Do the same for the high values. Depending on the 'Voltage Conversion Factor' selection, these values determine the conversion factor. At the very least however, they contain the hardware limits that will be checked in the course of the program to prevent damage. The default output units used in the program are grams and mm. Changing the hardware limits here will allow a change from these units to any user defined unit system with the exception that the main program display will still read g and mm respectively.

5.0 General Protocol Execution

A single protocol or protocol sequence (depending on whether program is in 'Run One' or 'Run All' mode) is initiated by pressing the 'Begin' button. When this button is pressed, a dialog windows pop up. In 'Run All' mode, a special dialog window pops up, as shown in Figure 6. The window sets the root filename extension of the series of data files to be saved during the protocol sequence. To select a different directory than the default, click on 'Get Data Directory' and another dialog box appears. Using the common methods, browse to the directory desired to hold the data files and press the 'Select Cur Dir' button. Filenames for each of the protocols are generated by taking the root file name provided in the 'Run All Protocols' dialog box and appending the number of the protocol currently being run.

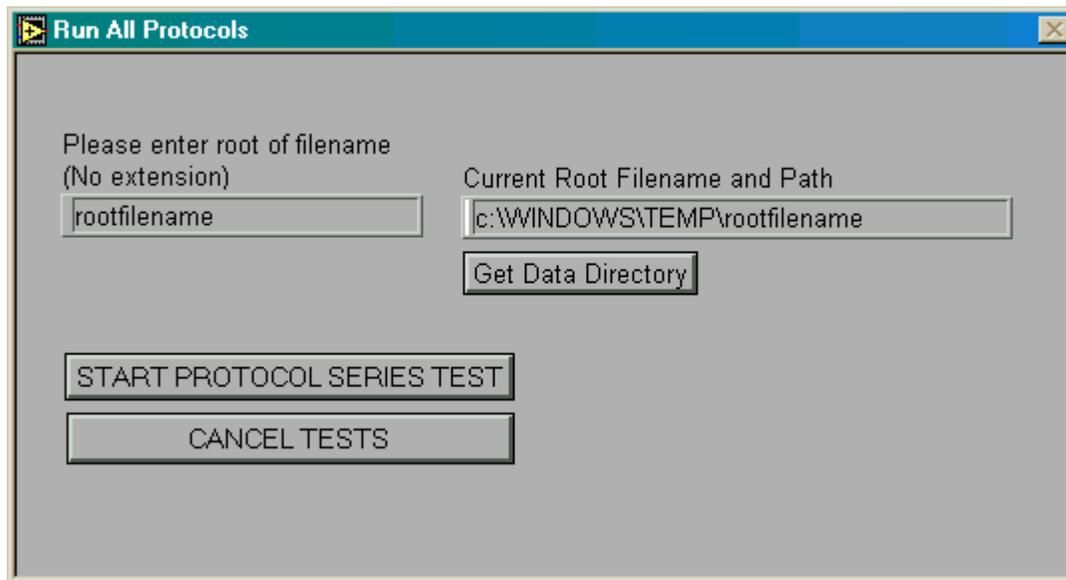


Figure 6. 'Run All Protocols' dialog box

Pressing the cancel tests button will return the user to the front panel without initiating any protocols.

After pressing the 'start protocol series test' button, a second dialog window is brought to the front. The window also pops up when initiating a protocol in the 'Run One' mode. The window is shown in Figure 7. The window allows a user to attach a descriptive string to a certain data file or set of data files. When finished entering the desired text, press 'done' to initiate the actual protocol execution or press 'cancel test' to return to the front panel of the DMC without executing any protocol.

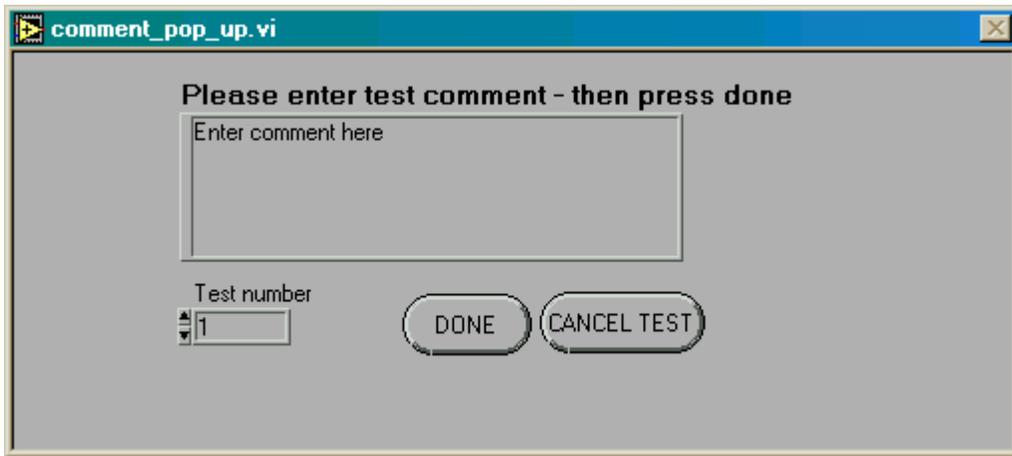


Figure 7. Comment dialog window

As soon as the 'done' button is pressed in the comment dialog box, the first protocol in a protocol sequence or the currently displayed protocol is executed depending on the mode of the program ('Run All' or 'Run One' respectively). The progress of an individual protocol can be observed in one of three ways. First, if the corresponding button in the monitoring control box on the main screen has been set to "yes", the data is displayed as it is collected, close to real time, as shown in Figure 8.

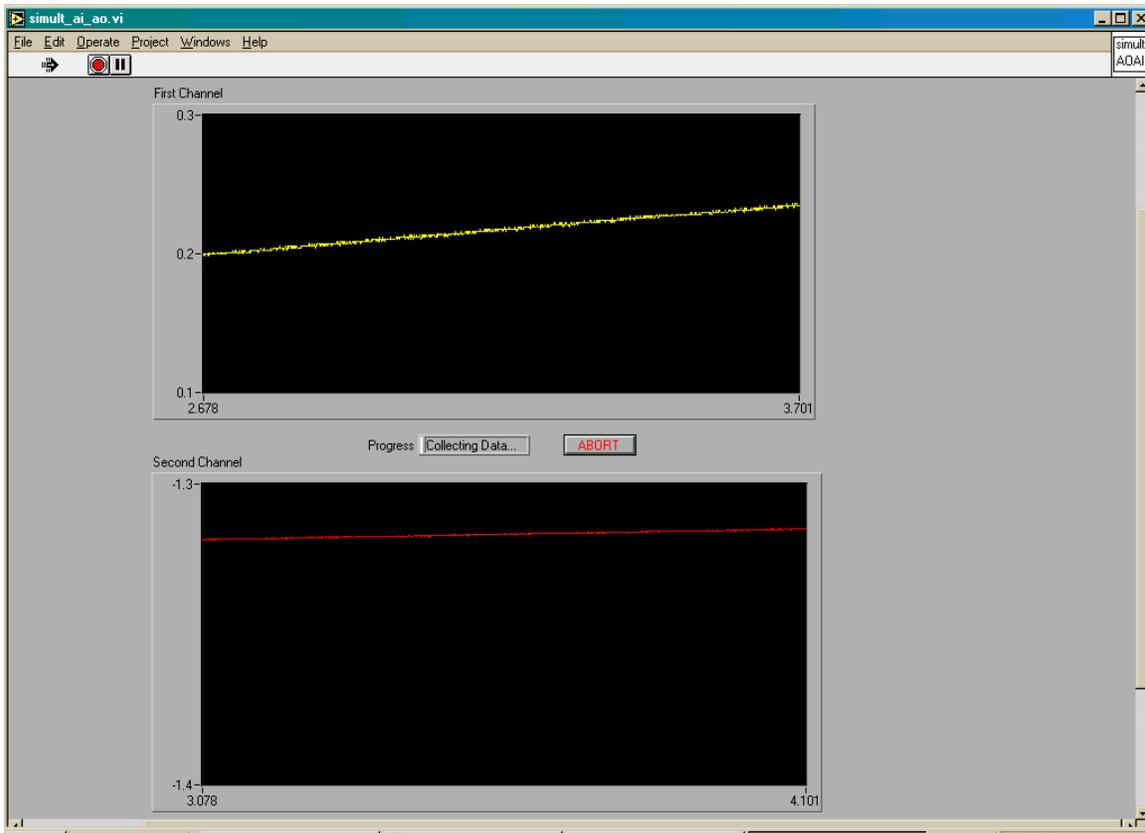


Figure 8. Data acquisition progress window - data shown

If real time monitoring is not enabled, the user will see a tank indicator filling up with data or merely an indication of the status of the acquisition for tests shorter than two seconds. The tank progress indicator is shown in Figure 9.

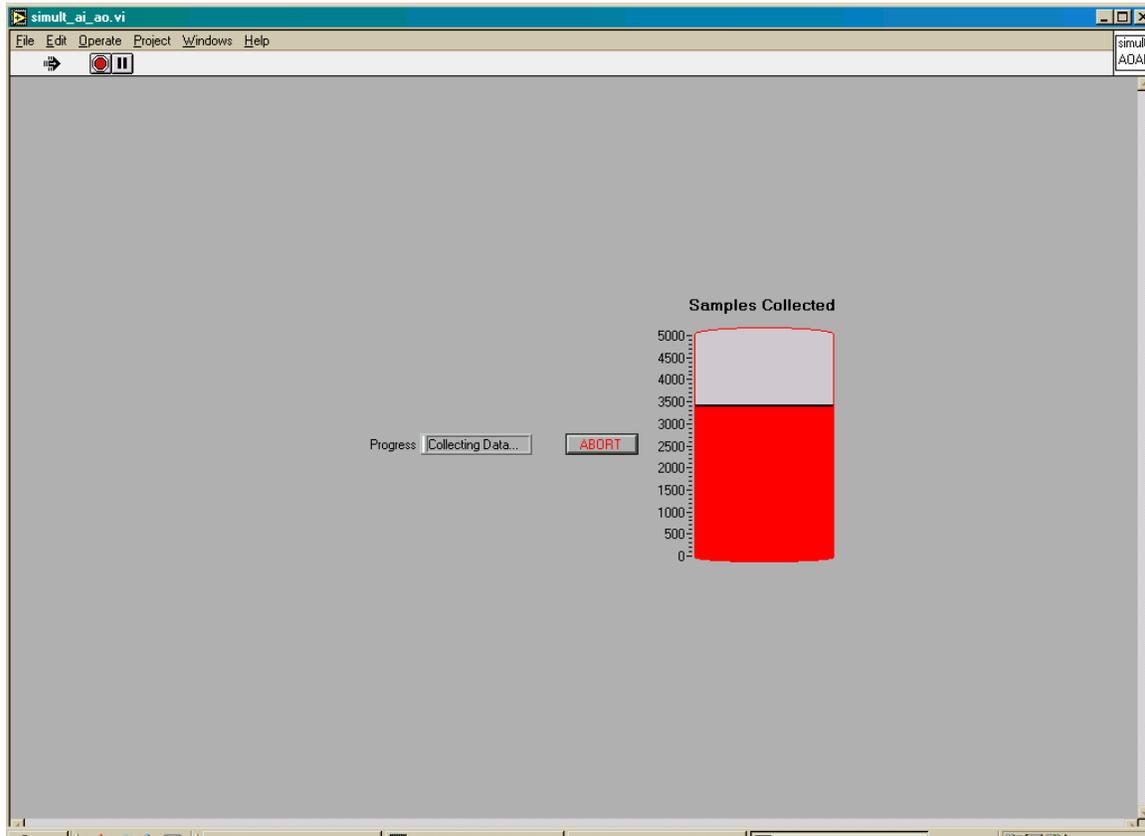


Figure 9. Data acquisition progress window - tank indicator

The lower the acquisition frequency, the closer to real time the display of data becomes. Regardless of the appearance of the display however, data is being continuously acquired at the user specified rate. If the program was in 'Run One' mode or there was only one protocol in the sequence, control will return to the DMC front panel with the collected data displayed in the data plots of the main screen. If more protocols follow, the next protocol is loaded and the program enters the monitor outputs window if the remaining wait time is greater than 5 seconds or the user has selected to control the start of the next protocol from this window. Figure 10 shows the screen for the case of user controlled protocol execution start. For the case where the wait time remaining is greater than five seconds, the window will show a timer counting down the remaining time, along with an abort button to immediately cancel this protocol and all remaining protocol execution.

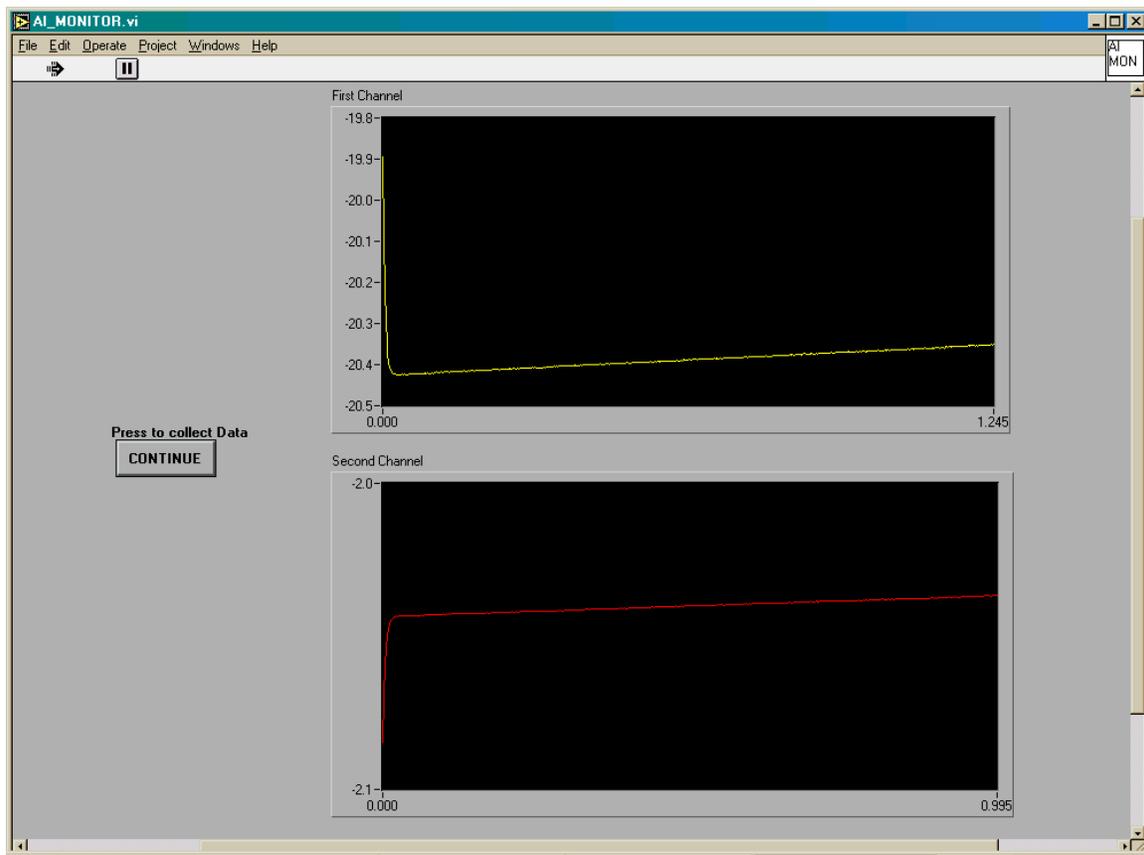


Figure 10. Data monitor window - protocol start control

If the remaining wait time is less than five seconds, the DMC front panel counts down the remaining time and then executes the next protocol. It is important to realize that the program begins to count down to the next test as soon as the last test has completed so that the waiting time display (in the main DMC or monitor windows) will not show the total desired wait time, but only what time remains after saving the last protocol's data and loading the next protocol test parameters. During protocol execution, the 'Erase Outputs', 'Erase All' and 'Browse / Export Data' buttons are replaced by the protocol counter, the delay timer and an 'abort' button. The data acquisition progress window will allow a test to be aborted immediately by pressing the abort button, regardless of the monitoring options or test length. Pressing the abort button in either the main DMC or monitoring windows during the delay time between protocols will result in immediate return of control to the front panel before the execution of the next protocol.

At the end of a protocol executed in 'Run One' mode, a dialog window is displayed to allow the user to enter the filename and location for the data file. The window is shown in Figure 8. After navigating to the desired directory, the user enters the filename with desired extension and presses 'OK'. Pressing 'Cancel' will discard the data and return the user to the front panel. The data can still be exported to ASCII using the 'Browse / Export data' button. The file dialog shown in Figure 8 is also shown when saving a protocol series, a calibration file or measurement setup file.

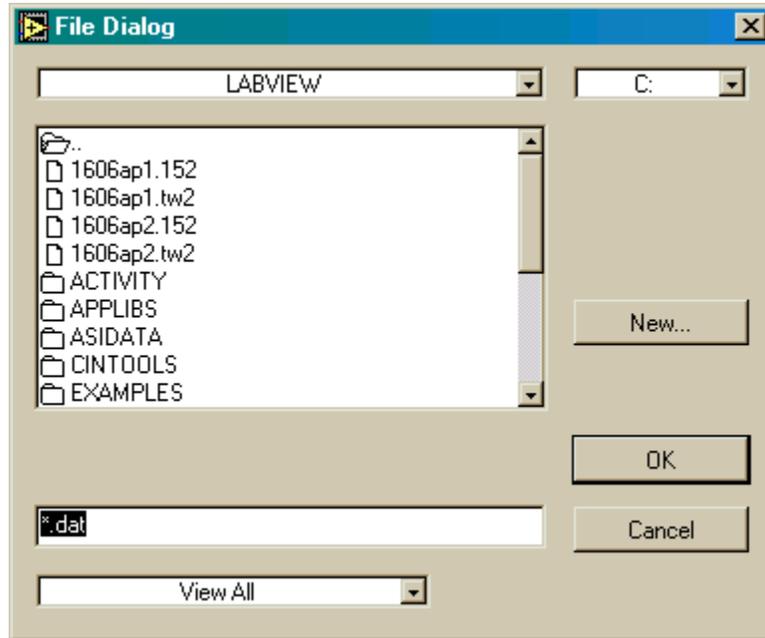


Figure 11. File dialog window

6.0 Stimulation Control

The following figures show the results of stimulation control. The results shown were obtained with an older version of DMC and therefore contain some differences in the front panel options. The controls of interest however, are the same.

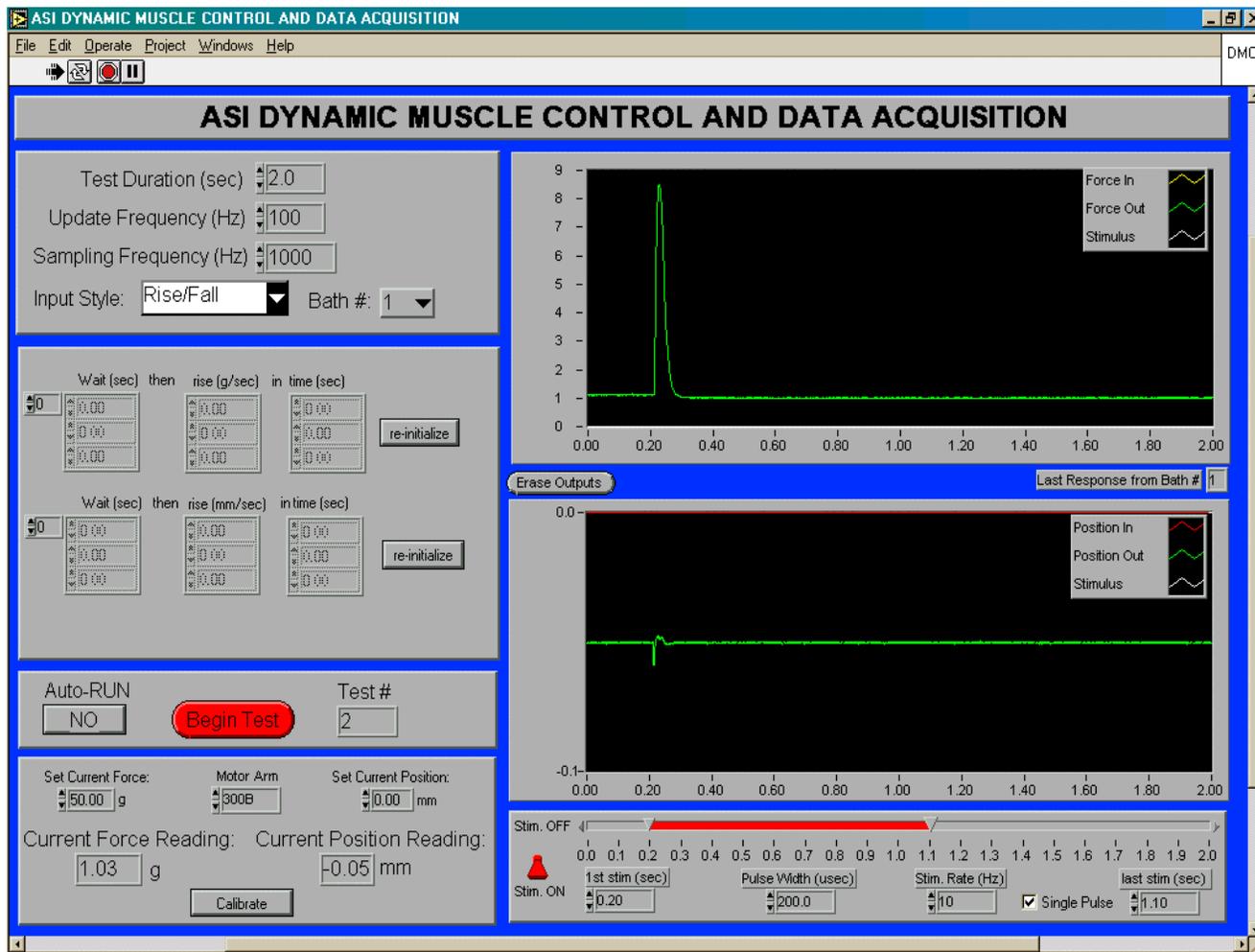


Figure 12. Single stimulation pulse response

Figure 9 shows an example of 'Single Pulse' mode. The 'Stim Rate' is set to 10 Hz but the 'Single Pulse' box has been selected by left clicking over the box with the mouse. A single pulse is delivered upon the execution of the protocol. and the 'Done' button pushed in the 'Comment' window that pops up.

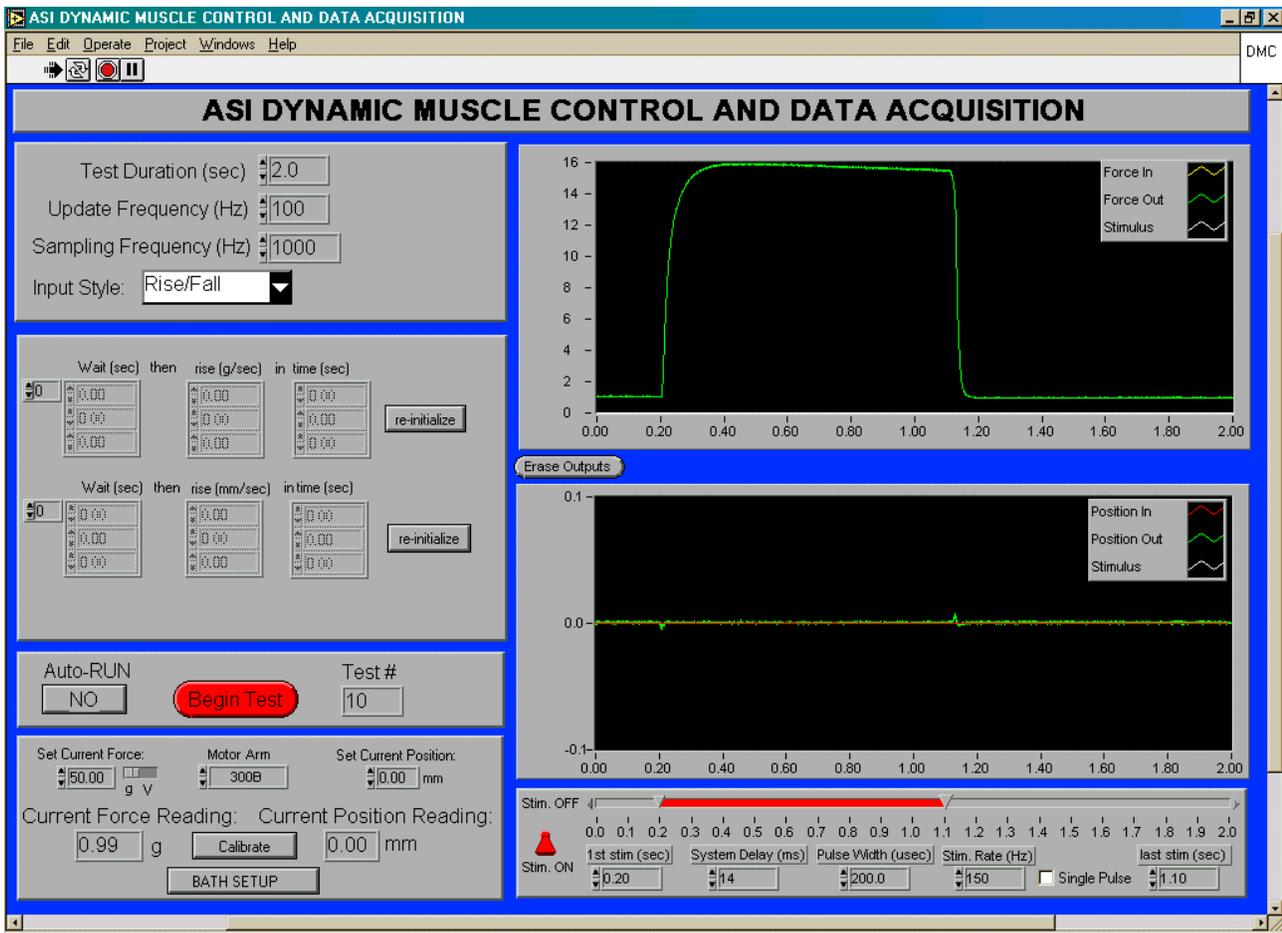


Figure 13. Example of pulse train stimulation

Figure 10 shows an example of a response from a mouse extensor digitorum longus muscle to a high frequency stimulation (150 Hz tetanus). The 'Stim Rate' is set to 150 Hz and the 'Single Pulse' box has been deselected by left clicking the mouse over it. A stimulus train at 150 Hz is delivered to the muscle for 0.9 s when 'Begin Test' is selected and the 'Done' button pushed in the 'Comment' window that pops up.

7.0 Calibration

The window shown in Figure 11 appears when 'calibrate' is selected on the front panel.

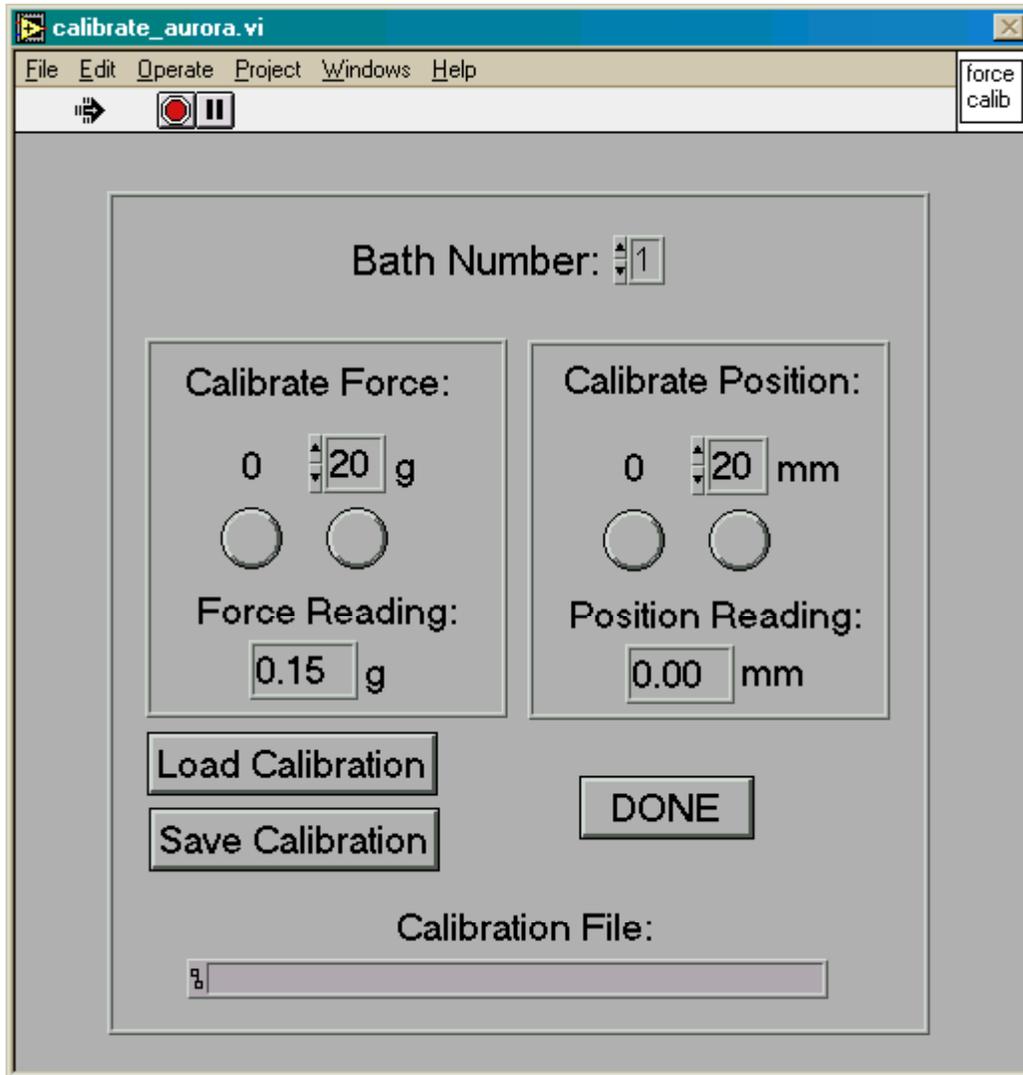


Figure 14. Calibration Window

- 1) All defined configurations can be calibrated individually
- 2) If a previous calibration has been saved it can be reloaded by pushing 'Load Calibration'. A **File Dialog** window appears for the user to select the correct file. Once selected, this file name is then displayed under 'Calibration File', and the calibration values are automatically loaded into the program. If the program is running two servo arms only one calibration file is saved for both (i.e., there are not separate calibration files for each servo arm, though the calibration values are different). When the user presses 'Done', the **Calibrate Window** disappears and the user is returned to the front

panel. If no 'Calibration File' has been saved previously for recall, continue as described below.

- 3) The mass for calibrating the range is usually hung from a rubber band. With the servomotor turned on, and only the rubber band on the arm, 0 g is calibrated simply by pushing the '0' button. The user can select the range in grams for the calibration by incrementing or typing in the desired value (1-X g; depending on the model servo motor). The mass corresponding to the Range reading (e.g., 20 g) is then hung from the arm with the same rubber band and the range button pushed (currently reading 20 g). The Range value can be changed by highlighting with the cursor and typing in the values, or by using the increment/decrement arrows.
- 4) The second servo arm can be calibrated in the same way, by selecting 'Bath Number' 2 (if equipped).
- 5) Length calibration using a suitable arm deflection measurement setup can be performed similar to the force calibration.
- 6) If one or both servo arms have been calibrated, the calibration data can be saved at this point by pushing 'Save Calibration'.
- 7) Save Calibration brings up a **File Dialog** window that prompts the user for a filename. When the file name is entered and saved, the user presses 'Done' and the **Calibration Window** will disappear. It is suggested that following each calibration, the data be saved, should it be necessary to reload the program. The calibration data are retained in the program if it is stopped (red button at top left of screen) and restarted (white arrow at top left of screen); therefore, reloading is not required.
- 8) When a muscle (or sample) is finally mounted on the transducer under no tension (i.e., slack), 'Calibrate' can be selected again, and the load on the arm under these slack conditions (e.g., weight of suture or other material used to tie the muscle to the arm) can be accounted for in the calibration by pushing the zero button again for the correct servomotor. The file can be saved again at this point. The user presses 'Done', and the **Calibration Window** disappears.
- 9) Resting tension on the sample preparation can be adjusted and monitored from the 'Current Reading' digital readout on the front panel of each module.
- 10) Note that the calibration functions available assume the sensors are linear. This is the case for ASI lever motors. Other linear sensors can also be calibrated here. However, non-linear sensor calibration and data interpretation must happen in post processing.

8.0 Individual Protocol Examples

The results shown here were obtained with a slightly older version of DMC. The examples can be transferred to the new version of DMC without difficulty.

8.1 Stretch Protocol

Figure 12 shows the results of a stretch protocol:

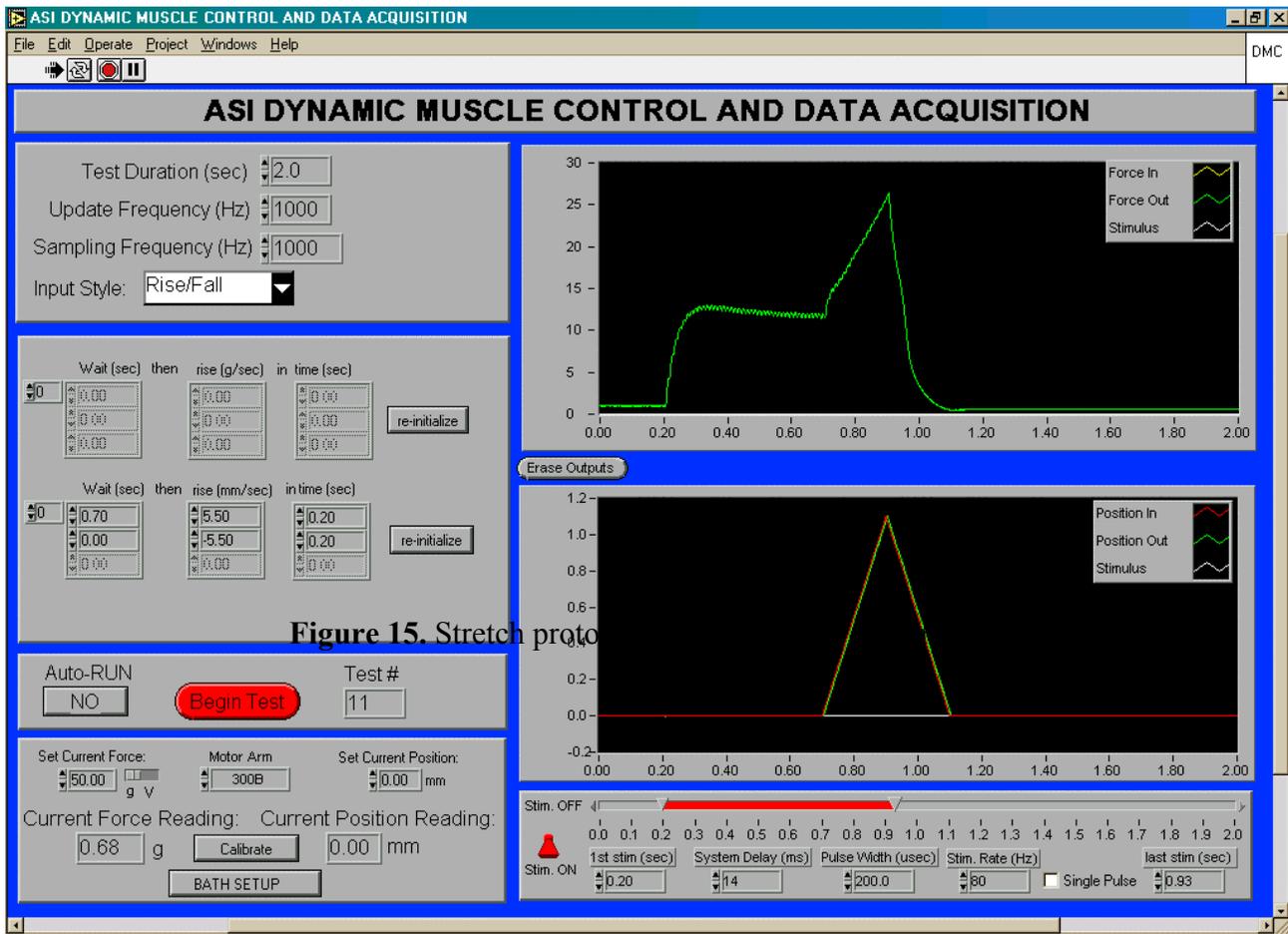


Figure 15. Stretch protocol

- 1) The settings as shown on the front panel yield a force profile as indicated.
- 2) This was a mouse extensor digitorum longus muscle stimulated at 80 Hz for 700 ms and then stretched at 0.5 muscle lengths per second for 0.2 s to yield a 0.1 muscle length stretch.

8.2 Work Cycle Study

Figure 13 is an example of using the sine wave module for a work cycle study. The module can also be used to obtain stress-strain data.

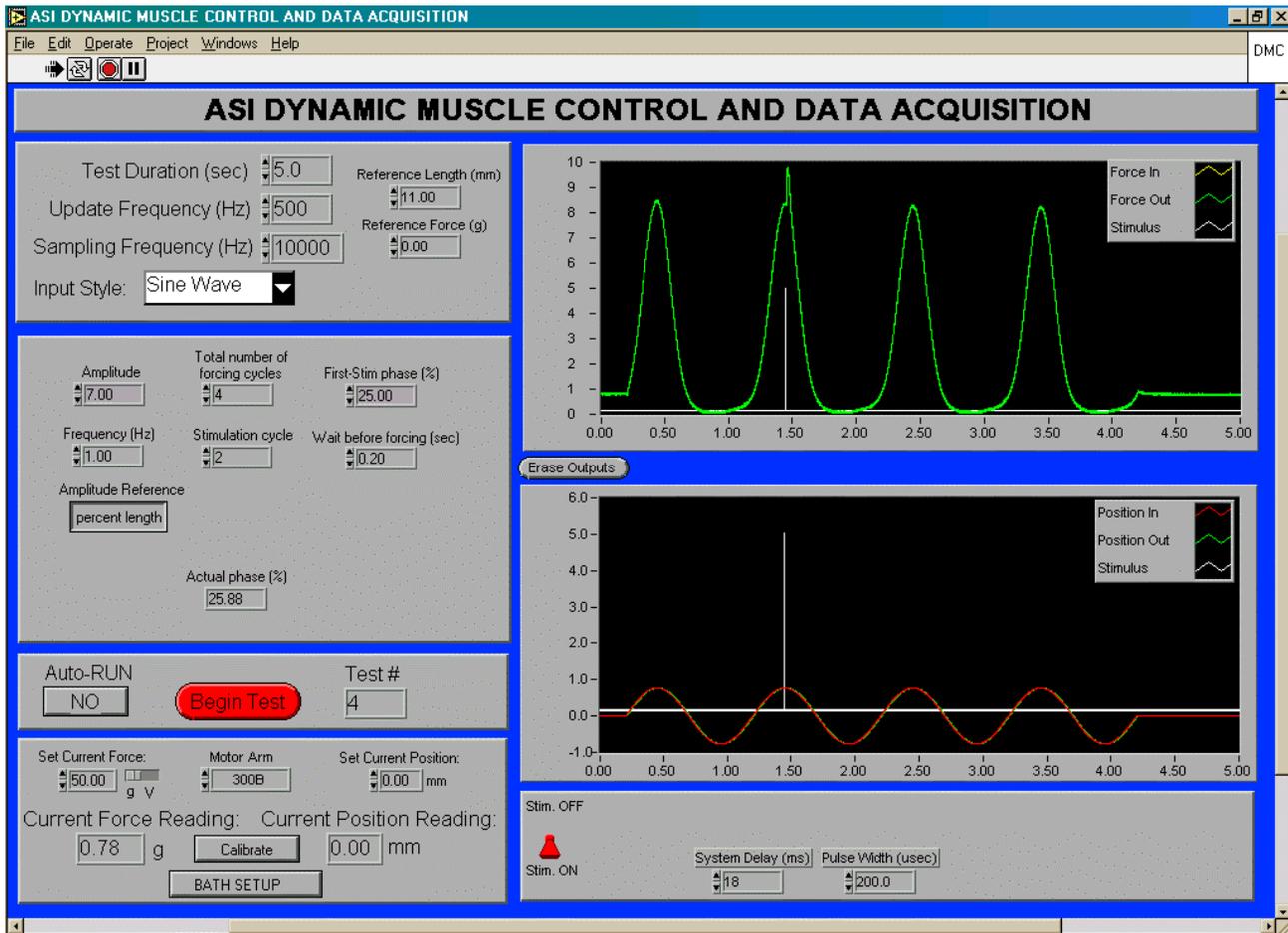


Figure 16. Work cycle study using Sine Wave module

- 1) The 'Test Duration' window is as described for Figure 12 except that the user can add a 'Reference Length' for the sample from which changes in length can be determined. 'Reference Force' is not functional in this module.
- 2) In the next window down, the user can enter 'Amplitude' in mm or as a percent of the 'Reference Length', enter the number of forcing cycles (1-200), enter the 'First Stim phase' in percent of sine cycle (see below) the sine 'Frequency' in Hz (maximum 20 Hz; suitable for most mammalian skeletal muscle preparations), the Stimulation cycle or cycle during which stimulation will be elicited, the 'Wait' before the sine function begins ('Wait before forcing'), and if the appropriate sampling frequency is selected (i.e., 10000 Hz for a 200 μ s pulse width) the 'Actual Phase' will be calculated (not shown). This calculation is included to account for minor (~1 ms) fluctuations in stimulus timing caused by the execution of the program. The user can control the location of the

stimulus start to within a ms, and the program can determine the actual stimulus location to within the accuracy of the sampling frequency used. For a 10000 Hz sampling frequency the timing can be determined to within a tenth of a ms. Note that the location of the stimulus will still be accurate to within a ms even if the sampling frequency is below the threshold for stimulation pulse display. The user can also select 'Amplitude Reference' as a percent of the 'Reference Length', or as absolute length in mm. For all inputs, the window can be highlighted with the mouse and values typed in, or the increment/decrement arrows can be used.

- 3) Accurate location of the stimulus can be assured by using an appropriate system delay value, entered in the corresponding box on the bottom of the screen. For the test configuration used here, a value of 6ms has been determined to work best. The system delay value for your system depends on the speed of your PC, the memory capacity, other programs running and to a lesser degree on the length of the test.
- 4) Amplitude (mm). Maximum value depends on servomotor model. Minimum value depends on resolution of the servomotor model.
- 5) The 'Total number of forcing cycles' (no.) will repeat the sine wave for minutes to hours. However, this means the data file could be quite large depending on the sampling frequency. One alternative, if it fits with the experimental protocol, would be to use a protocol series to separate each stimulation cycle.
- 6) First-Stim Phase is set by the user as a percentage of the sine cycle and determines when in the specified cycle (i.e. Stimulation cycle), the stimulus will occur. A complete cycle is 100 %. The beginning of the sine wave is 0%, the first peak is 25%, return to baseline is 50%, the nadir is 75% and the return to baseline is 100%. To stimulate at the peak of the sine wave, i.e., at the longest length of the muscle, a phase of 25% would be selected. The user can select any phase to initiate stimulation in unit percent values between 1 and 100%.
- 7) For comparing passive and stimulated responses, the program can be set up to produce a pair of sine waves at the given frequency and amplitude. This has been done so that the response of the muscle preparation without stimulation is captured prior to the stimulated response. The difference between the two responses is the active response of the muscle.
- 8) Maximum and minimum frequency settings are limited by the specifications of the servo motor model.
- 9) In the Stimulation Window, only Pulse Width is displayed. Some early versions of the program only support single pulse stimulation. Some of the following examples will deal with the updated ability of the program to provide multiple pulses during sine function position protocols.
- 10) The settings are indicated in the screen shot. Note that the 'Amplitude' is expressed as 7% of 'Reference Length' (11 mm), because 'Amplitude Reference' is 'percent length'.
- 11) The 'Update Frequency' was set at 500 Hz and the 'Sampling Frequency' set at 10000 Hz. With a high sampling frequency, 'Actual Phase' is also displayed. There is some discrepancy between the desired 'First-Stim phase' and the 'Actual Phase'.

8.3 Multiple Pulse Stimulation During Sine Wave Execution

What follows is an example of the (DMC) program with revised sine wave module allowing for multiple pulses of stimulation to be generated during sine wave execution.

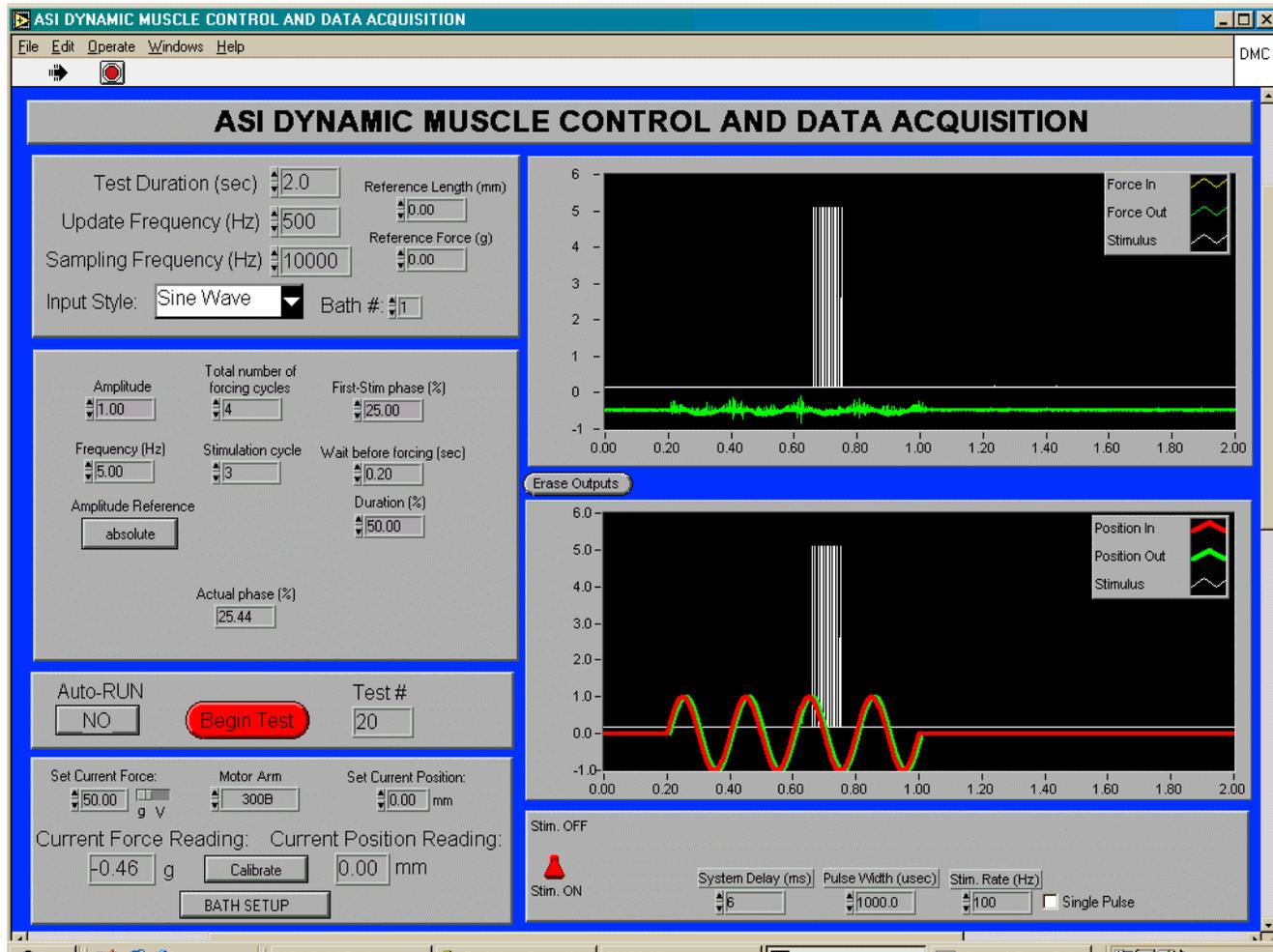


Figure 17. Multiple pulse stimulation during sine wave execution

- 1) The latest revision to the Sine Wave module of the DMC permits greater flexibility in selecting the frequency and duration of stimulation during the sine profile of interest.
- 2) Variables were input as is shown above in Figure 14.
- 3) There was no load on the arm (i.e., no tissue, no rubber band etc.) in these screen shots.
- 4) The line thickness for the 'Position In' and 'Position Out' were changed so they would be more visible (simply Right click on the mouse with the pointer over the desired line in the upper right corner of the Position Display, then select 'Line Width' (Left Click mouse) from the pop up menu, and select the desired width from those displayed in the window.
- 5) Figure 14 shows the timing and amplitude of the sine wave that will drive the movement of the arm ('Frequency (Hz)', currently 5 Hz), as well as the onset of stimulation ('First

Stim Phase (%)', currently 25%), the duration of stimulation ('Duration (%)', currently 50%), as well as other parameters as shown. Note that the stimulation duration is input as a percent of the sine cycle (e.g., 50%); therefore, the stimulation will begin at the peak of the 3rd sine wave and continue to the nadir of the same sine wave (i.e., 50% of the total sine cycle). To calculate the number of stimulation pulses during this time, first determine the duration in ms relative to the sine frequency ($\text{Duration}\% \times 1/\text{frequency}$), where sine frequency is expressed in sine waves per second, and then multiply this value by the stimulation frequency. In the example, $50\% \times (1/(5 \text{ pulses}/1000\text{ms}))$ (i.e., $50\% \times 200 \text{ ms}$) = 100 ms; $100 \text{ ms} \times (100 \text{ pulses}/1000 \text{ ms}) = 10 \text{ pulses}$.

- 6) Stimulation frequency is set in the 'Stimulation Window' at the bottom right of the screen. Note that both minimum 'Pulse Width' and therefore maximum stimulation frequency may be limited depending on the stimulator being used. Check your hardware specifications for this information.
- 7) 'System Delay (ms)' is unique to each computer and should be determined empirically. Varying the value in this dial will influence the 'Actual Phase (%)' calculation, such that the calculated value will be either closer to the desired 'First Stim Phase (%)' setting, or further away. The current 'System Delay (ms)' setting is 6 ms, but this will likely be different for each system that is running the DMC software; therefore, make adjustments as necessary. Simply run the same parameters for the sine function and vary the system delay value and note the Actual Phase (%) value until satisfied.
- 8) The vertical white lines in Figure 14 indicate the timing of the stimulation pulses.
- 9) The 'Actual Phase (%)' has been calculated as 25.44% compared to the desired 'First Stim Phase (%)' of 25%. The error is less than 0.44% of the sine period ($0.44\% \times 200 \text{ ms} = 0.88 \text{ ms}$).

8.4 Force-Velocity Curve

Figure 15 shows an example of the use of the 'Muscle %' module. The protocol is set up to wait 0.40 sec and then move to 25% of 20g (Reference Force), or 5 g. After maintaining this load for 0.1 sec, the force is ramped back to 250% of 'Reference Force', or 50 g. This approach can be used to perform a series of tetanic quick releases to generate a force-velocity curve. Note: the 'Reference Force' in this example was set arbitrarily to 20g, but is typically set at the maximum force for the muscle. As shown in figure 15, the maximum for this muscle was actually ~13.5g.

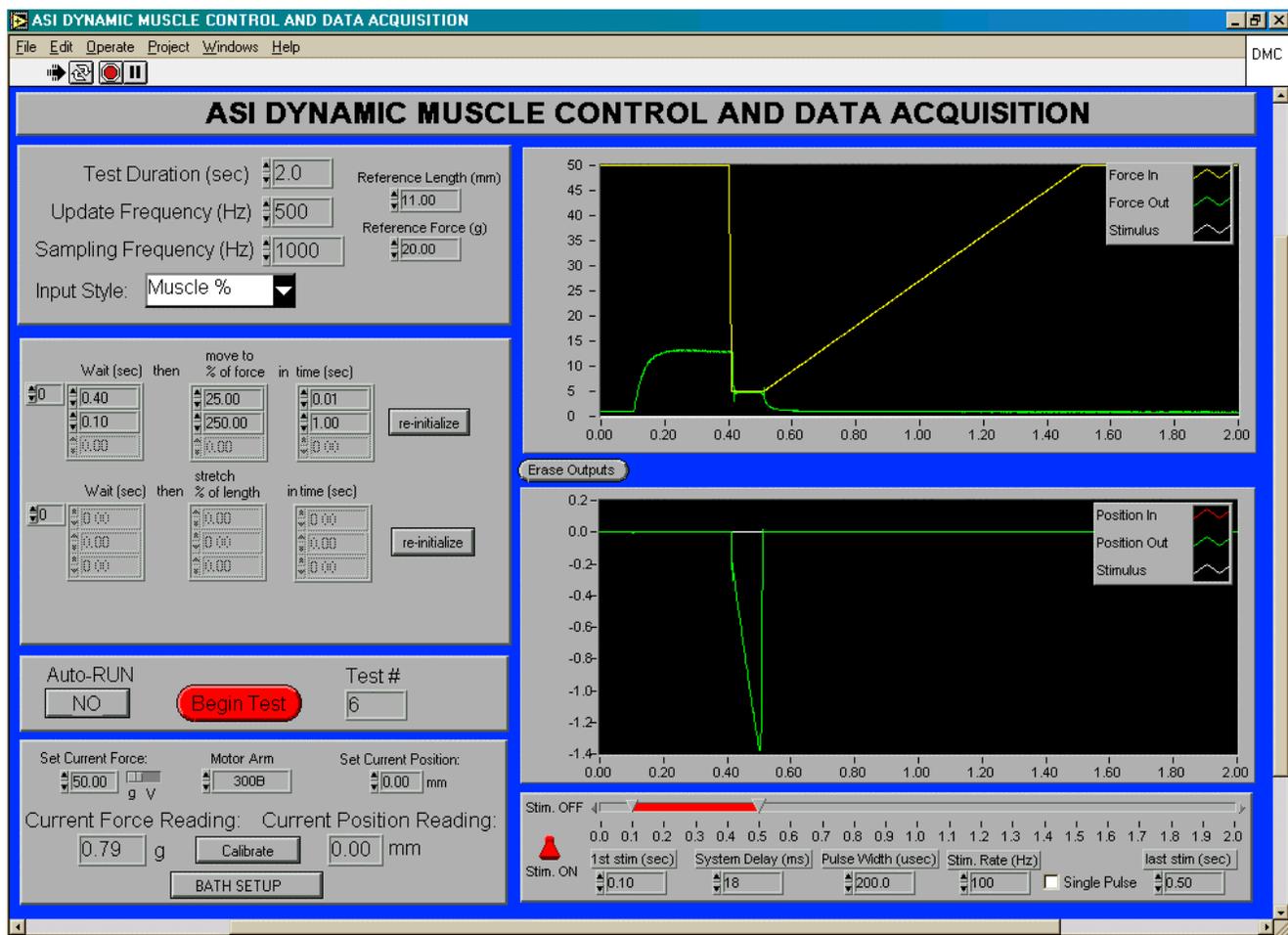


Figure 18. Example using Muscle % module

Mouse extensor digitorum longus muscle stimulated isometrically at 100 Hz for 0.4 sec and then 'released' to 5g for 0.1 sec. The shortening of the muscle is shown in the bottom display.

8.5 Tetanic Afterload

Figure 16 shows an example of Tetanic Afterload.

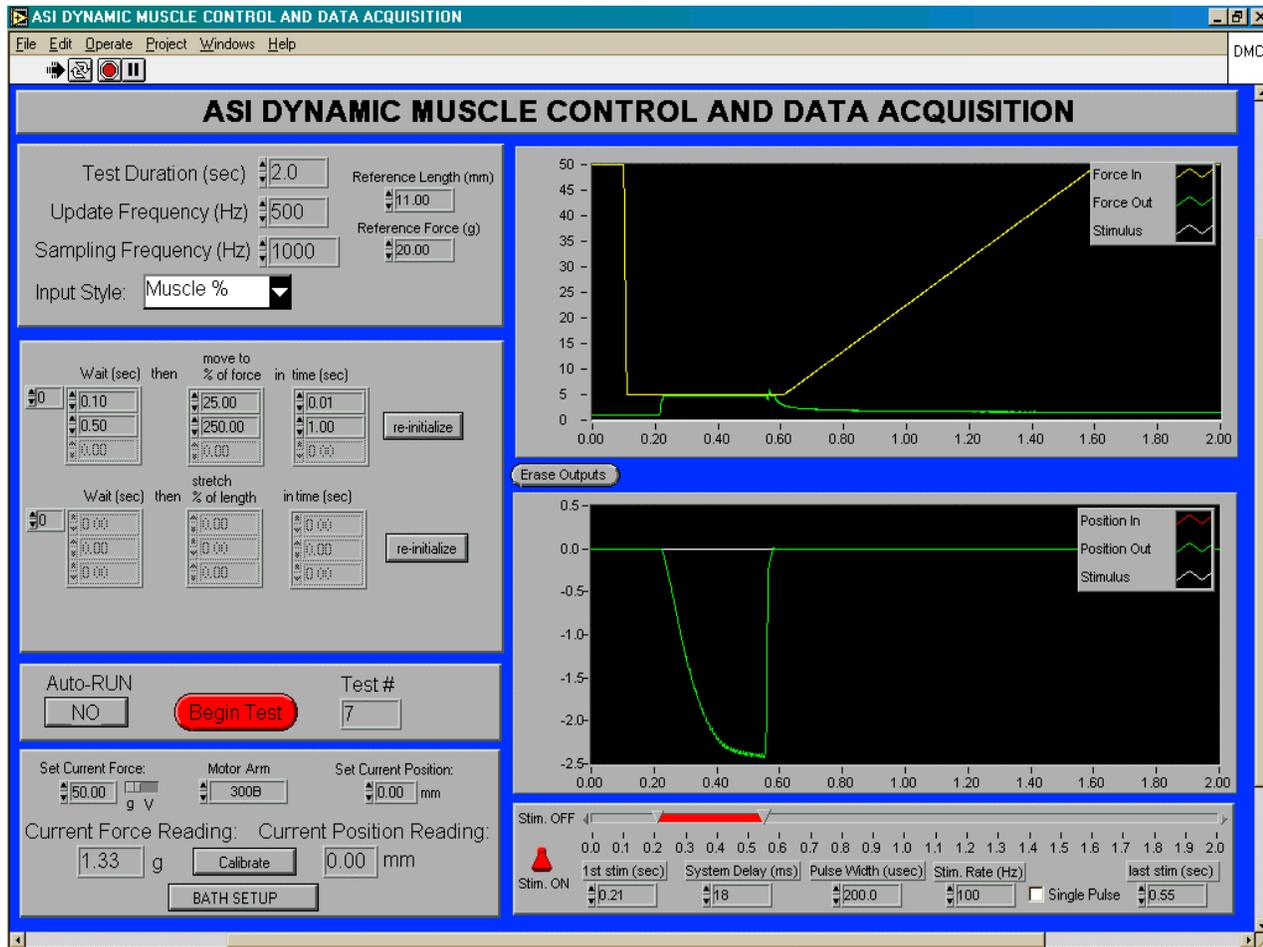


Figure 19. Tetanic after load using Muscle % module

The settings demonstrate a change in load on the arm to 5 g (25% of 20 g Reference Force) before stimulation of the mouse extensor digitorum longus muscle. When stimulation is initiated at 100 Hz, the muscle contracts isometrically until the muscle force exceeds the load on the arm (i.e., > 5g), at which time the muscle then shortens (lower display) at a load of 5 g (upper display). This is known as an afterloaded contraction. By employing a series of loads in this manner, a force-velocity curve can be derived.

8.6 Strain-Relaxation Test with a Rubber Band

Figure 17 shows an example using the Muscle % module in a strain-relaxation test for a rubber band:

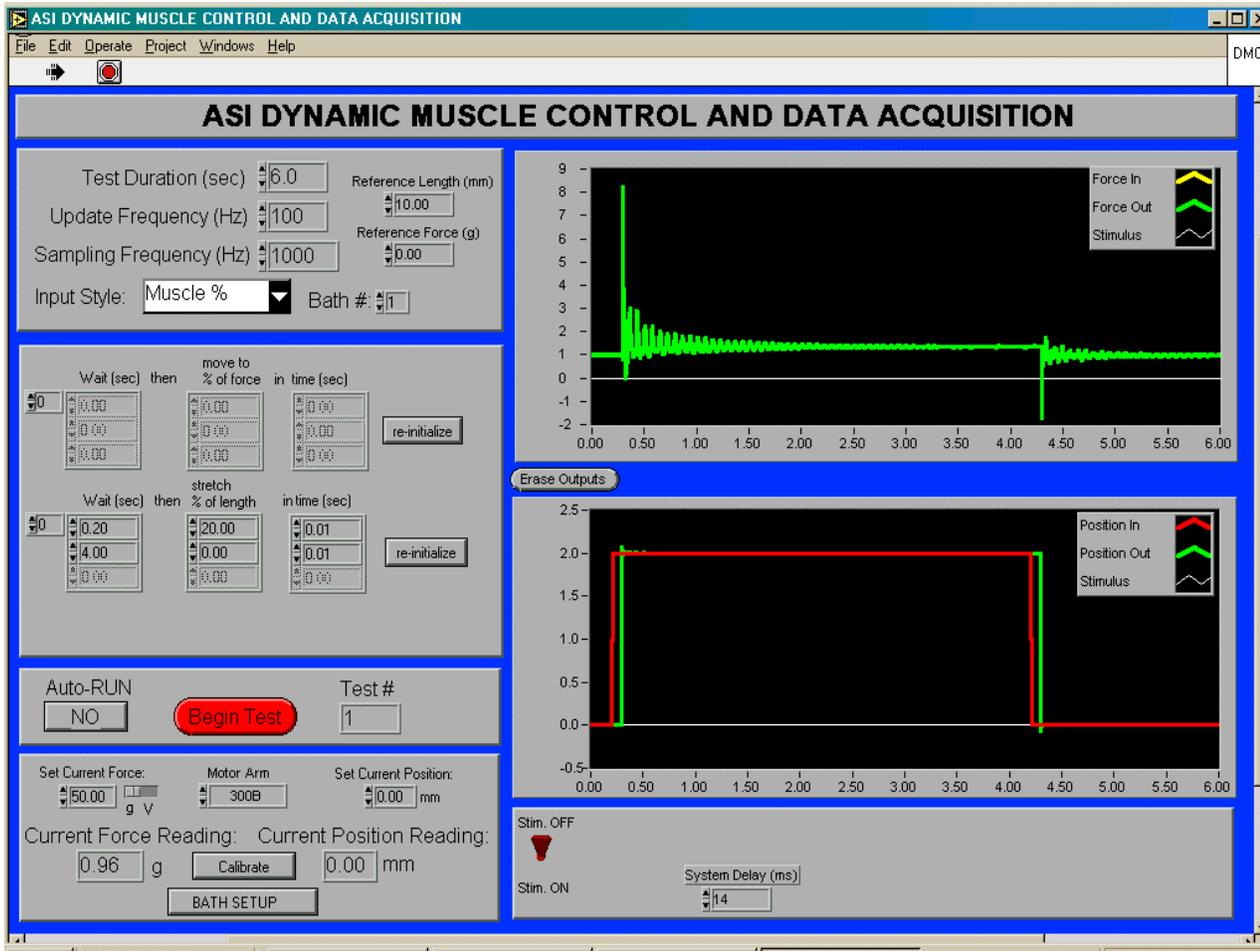


Figure 20. Strain-relaxation example using Muscle % module

1. In the **Muscle% Module**, a 'Test Duration' of 6.0 s, a 'Muscle Length' of 10.0 mm, an 'Update Frequency' of 100 Hz and a 'Sampling Frequency' of 1000 Hz have been entered.
2. The change in length as a percentage of the 'Muscle Length' (i.e. strain) is entered in the bottom half of the next window. A 'Wait' of 0.2 s, a 'move to % length' of 20%, in 'time' 0.01 s are entered in the first row (i.e., wait 1 s then move arm position 20% of Muscle Length, or 2 mm).
3. The second row has 'Wait' of 4.0 s, 'move to % length' of 0% in 'time' of 0.01 s (i.e., hold arm position for 4 s then return to original Muscle Length).

4. Note that the red line in the Position display reflects the **Position input** to the servo arm; after running the routine, the green line reflects the **Position output** from the servo arm. The green line in the Force display reflects the **Force Output** of the rubber band after the routine is run. The green and red lines are offset slightly in time because the start of data acquisition does not exactly correspond to the start of analog output control. Stimulation and analog output are very tightly controlled in terms of timing with respect to each other. The timing difference between the start of data acquisition and the start of analog output is usually much less than 0.1 sec and therefore can be easily accounted for in the specification of wait times and test duration.
5. Caution: Be aware of the duration of the routine and the 'Update' and 'Sampling' Frequencies. The update frequency is used for the Position Display. If a duration of several minutes (e.g., 120 s) is entered and the update frequency is 1000 Hz, then 120,000 sample points will be displayed and stored with the data file. Similarly for the Sampling frequency, the file size can be very large depending on the frequency selected and test duration.
6. The time period over which a change in length or force is specified must be commensurate with the update rate in order to be accurate. For example, in order for a length change to actually occur over 0.01 seconds, the update rate must be at least $1/0.01 = 100$ Hz.
7. Set – up and execution:
 - a. A rubber band was fixed at one end and the other end connected to a hook on the servo arm under slight stretch.
 - b. The values for the routine were entered as described above.
 - c. 'Begin Test' was selected and then a comment placed in the 'Comment Window'.
 - d. In the 'Comment Window', 'Done' was selected to initiate the routine.
 - e. At this point the program will display the data collection monitoring window with the actual data being collected (Figure 8) or a data acquisition progress indicator (Figure 9) being shown. Which display is shown depends on the user options entered in the front panel monitoring controls for the present protocol.
 - f. At the conclusion of the routine, the Force and Position Outputs are displayed and a 'File Dialog Window' pops up.
 - g. The user can drag the 'File Dialog Window' away from the displays to view and decide if the data should be kept. A drive and folder can be selected in the 'File Dialog Window' or a new folder created.
 - h. The user can enter the appropriate file name.
 - i. Alternatively, the user can cancel and repeat or alter the routine. The currently displayed outputs can be erased by selecting the 'Erase Outputs' button between the Force and Position displays.
7. To change the settings for the routine, the user can go to the desired input and highlight with the cursor and then type the values, or use the increment/decrement arrows. Note that several steps in strain can be entered in the same routine. However, remember to take into account the duration of the overall test and the Update and Sampling Frequencies, as these will determine file size.

8.7 Strain-Relaxation with Mouse Extensor Digitorum Longus Muscle

Figure 18 shows a strain-relaxation example with mouse extensor digitorum longus muscle.

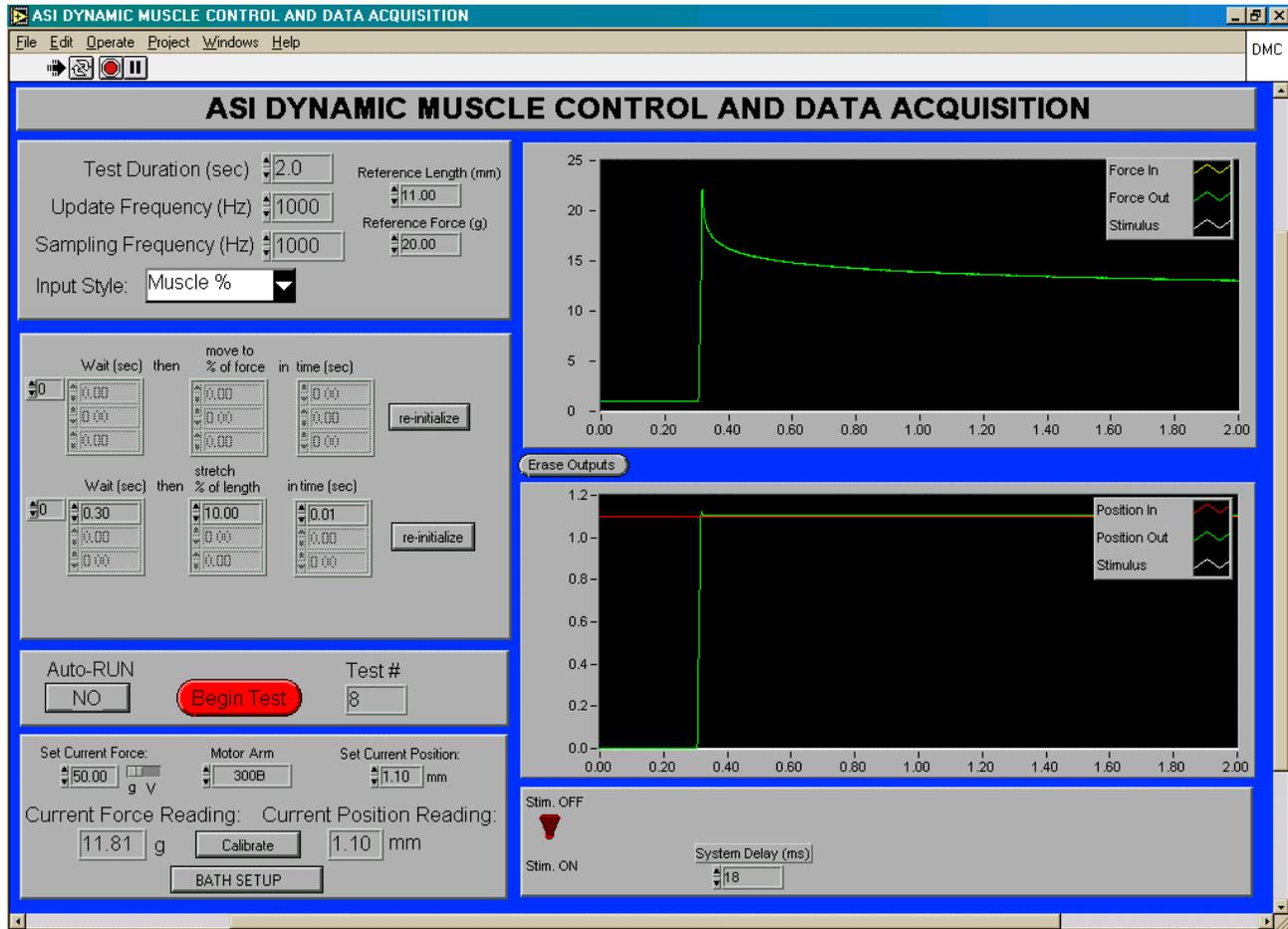


Figure 21. Muscle strain relaxation example using the Muscle % module

- 1) The muscle is stretched 10% of its Reference Length (11.0 mm), or 1.05 mm in 0.01 sec. Note the arm position is not programmed to return to its original position, and therefore remains at 1.05 mm from its starting point of 0 mm (neutral position). Note also that this is a passive perturbation; the muscle is not stimulated.
- 2) The total 'Test Duration' is 2 s; therefore, 2 s of data are collected to follow the relaxation from the imposed strain. Duration can be increased as necessary.

9.0 Browse / Export Data window

Figure 19 shows the browse and export data window.

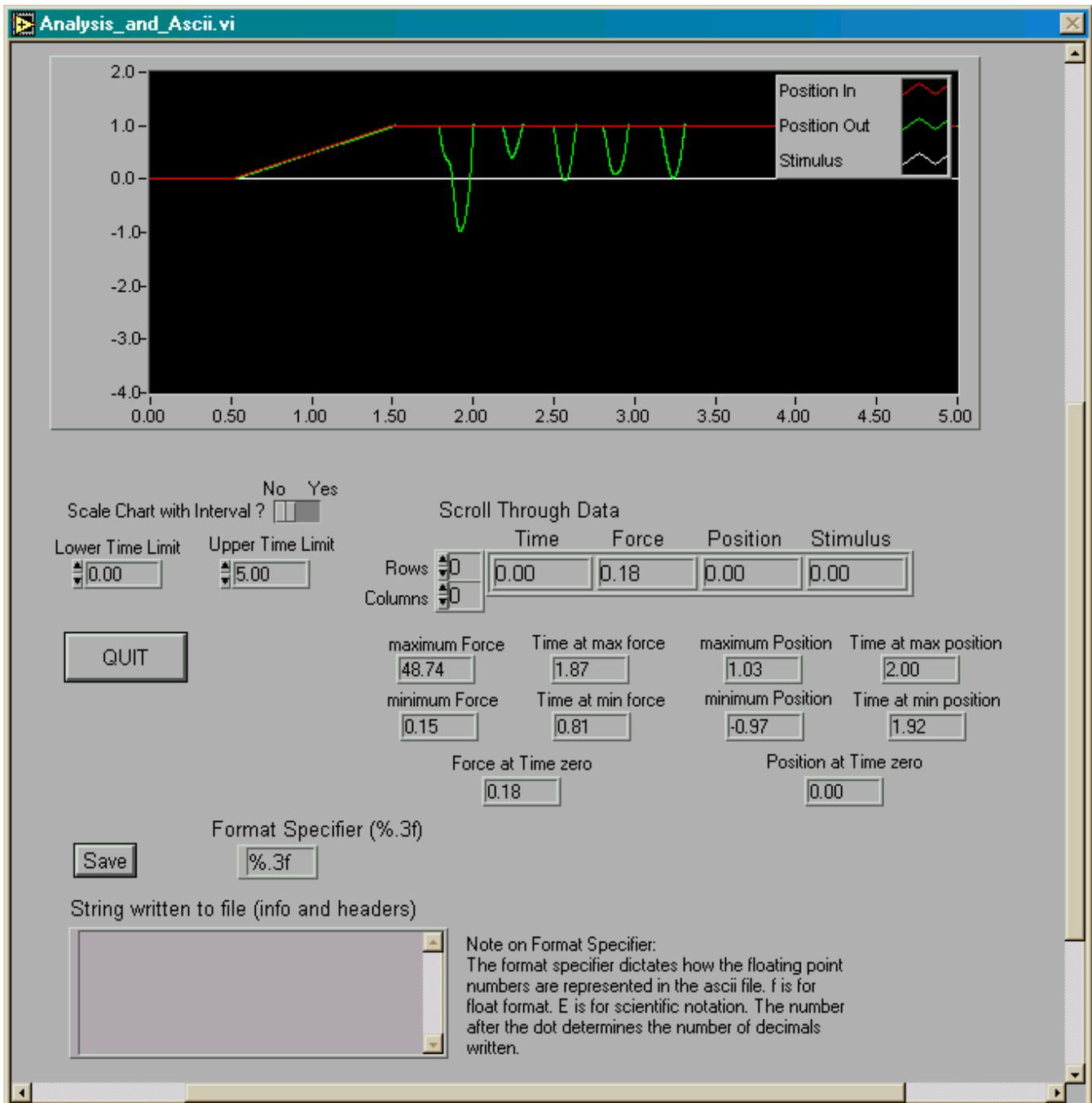


Figure 22. Browse / Export data window

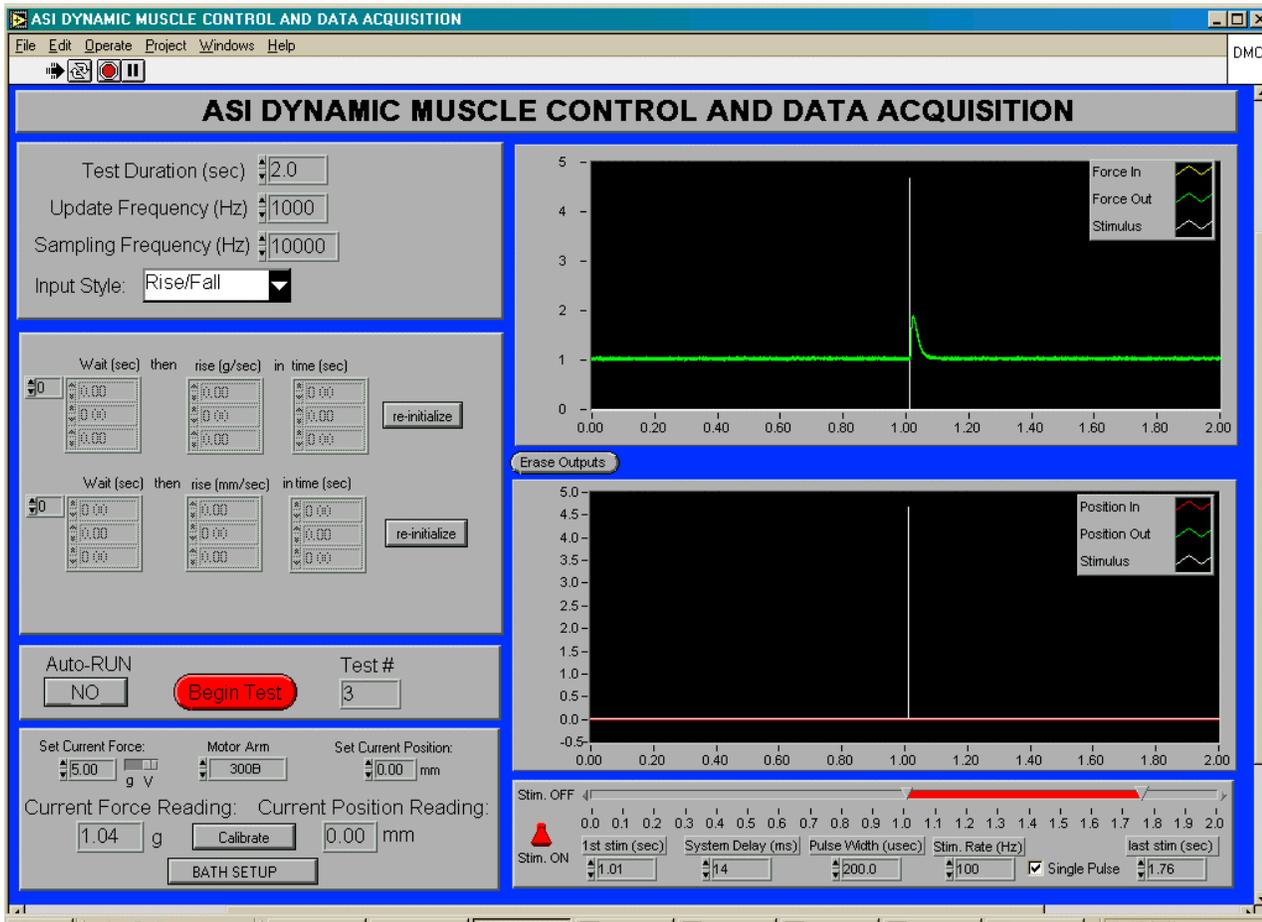
The window is called by pressing the 'Browse / Export Data' button between the force and length graphs. Both graphs are also accessible in the 'Browse / Export Data' window, by using

the scroll bar on the right side of the window. In this window, the user can zoom in on selected data using the lower and upper time limit controls. These controls will update the chart if the slide switch is set appropriately. Otherwise, the data within the selected limits can be looked at individually by clicking on the up and down arrow beside the row control. The maxima and minima of the data along with their times of occurrence are also given for the interval selected. The values at time zero are the first readings in the selected interval.

The data for the selected interval can be exported to an ASCII output file by pressing the 'Save' button. The format specifier determines the format of the stored data. This specifier is explained in the window and largely follows traditional programming language conventions. A location is provided to write out headers for the data as well as a description of the data. The text string is inserted before the written out data. Note that only the data between the upper and lower time limits will be saved to the ASCII file.

10.0 Controlling Stimulation Voltage

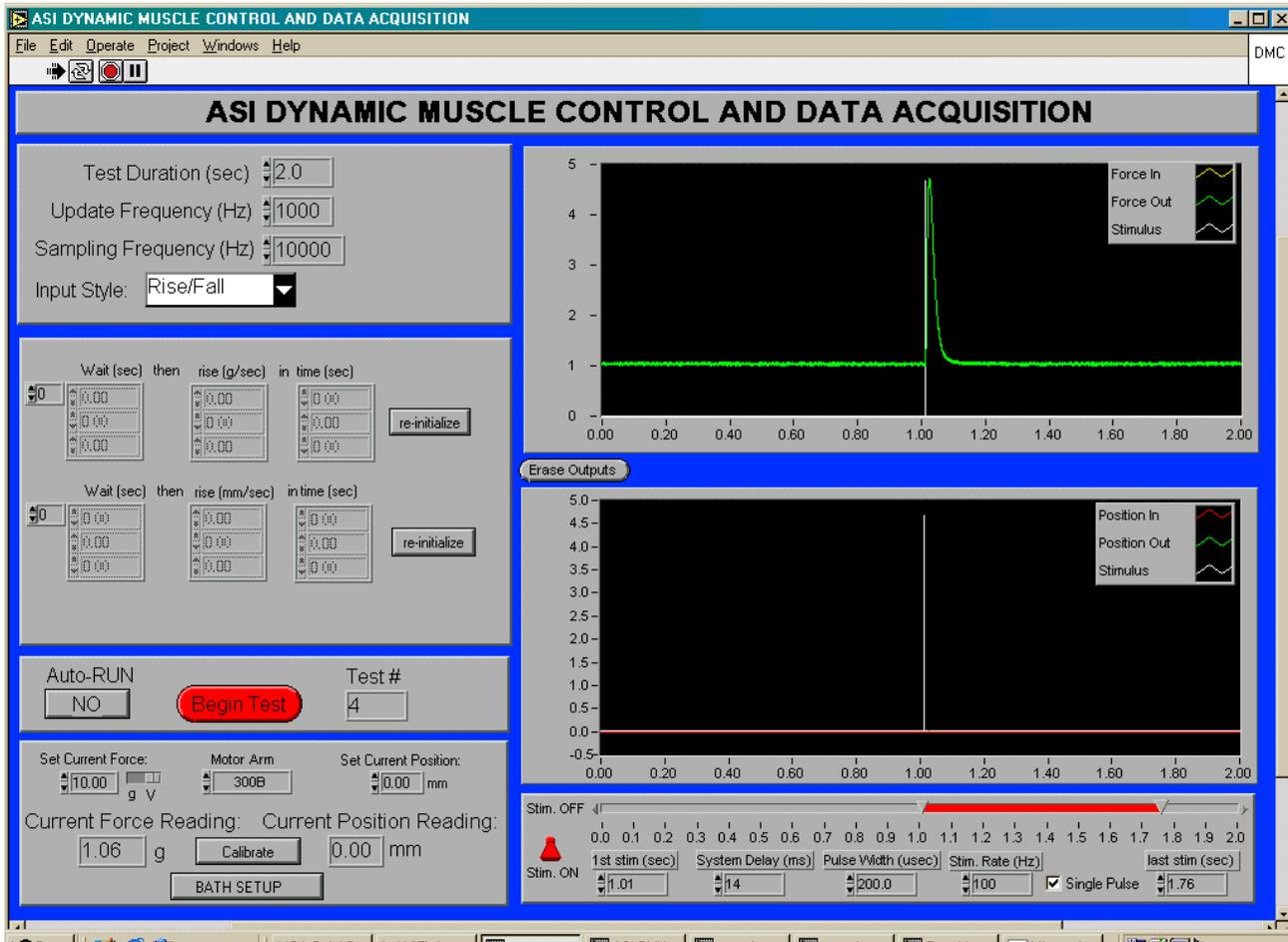
The remaining pages of this manual are dedicated to a feature available only if force control is not needed. In that case, the additional analog output can be used to control stimulation voltage when an appropriately controllable stimulator is used. It is also possible with proper hardware to control the stimulation voltage using the digital port on the data acquisition card. The stimulation voltage can be controlled using different bath definitions and concurrent different digital outputs on the data acquisition card. Contact ASI for more information.



Screen shots for the Dynamic Muscle Control (DMC) program.

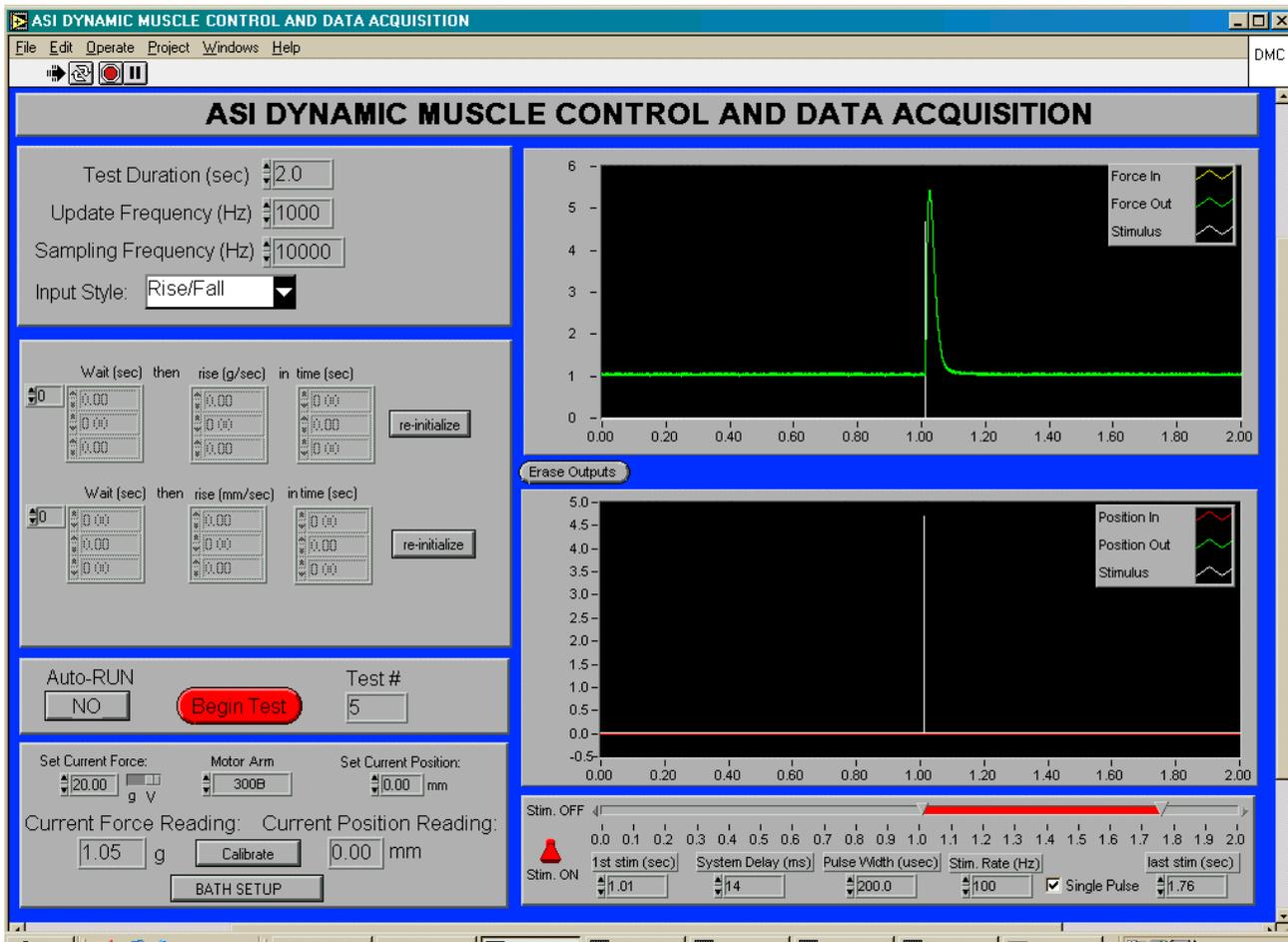
Stimulation Voltage Control. In the case where force control is not needed or desired, the force voltage output from the card can be converted to a stimulation voltage controller. Currently, the software is setup to provide a 0-5 V volt output linear with 0-125V stimulation voltage. On the front panel stimulation voltage form 0-125 V is entered in the 'Set Current Force:' dialog box. Before entering the desired stimulation voltage, please assure the slider switch shown just above the **g / V** characters is set towards the **V** character. To change to the **V** setting, click in the dark area with the mouse pointer just to the right of the current slider position. To switch back to force control, once again click in the dark area that is now to the left of the slider switch.

The present slide and the following two slides demonstrate the effect of different voltage settings on the contractile amplitude of a mouse EDL muscle. The screen shots were taken at increasing voltages of 5, 10 and 20 V.



Screen shots for the Dynamic Muscle Control (DMC) program.

Stimulation Voltage Control. (cont.) 10 volt output from stimulator.



Screen shots for the Dynamic Muscle Control (DMC) program.

Stimulation Voltage Control. (cont.) 20 volt output from stimulator.