Operators Manual

3-Point Bending Device to Measure Transmural Strains for Multilayer Soft Tissue Composite

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Important Safety Instructions:

This device has been constructed to be used in the laboratory of Dr. Wei Sun. It consists of many parts that have the potential to cause bodily or environmental harm if not handled correctly. Below are a list of the individual parts and the dangers involved with them.

- Stepper motor – Rotating shift may cause damage if long hair or loose clothing comes in contact
- Linear actuator – Potential to pinch body parts if placed in the way of the moving piece
- Temperature regulator/ Outer bath – Leaking fluid may occur if hose is not secured properly
- Bending Bar/Inner Bath – Constant contact with live specimens can lead to bacterial growth
- Chemical Medium – Phosphate Buffered Saline (PBS) enhances corrosion in many materials

Preventative measures were put in place to minimize dangerous components of the device. Because the stepper motor was much larger than the linear actuator, a piece was fabricated to couple the two components together. Another aluminum piece was screwed to the linear actuator and into our testing board so that the linear actuator was kept in place and the stepper motor was lifted slightly off of the ground. This system prevented the linear actuator or stepper motor from moving around on the testing surface and enhancing any potential for harm.

The temperature regulator has all components enclosed. Tubing is thoroughly attached to the regulator so that fluid cannot leak out. The design of the regulator also prevents fluid from coming in contact with any of the electrical components. The chemical medium was chosen to resemble human bodily conditions. Coming in contact with PBS is minimally harmful. The only negative of PBS is that it may catalyze corrosion in other materials of the device. It is extremely important to clean materials in contact with the PBS thoroughly after each trial and to have spare components if economically permitting.

The testing area on the surface of the inner bath provides another health concern. Through the design of the bath within a bath, many of these concerns are negated. Live tissues will be used on the surface of the inner bath which will lead to bacterial growth. By having the inner bath separate from the constantly regulated outer bath, the bacteria cannot spread throughout the entire system. It is also important that the inner and outer bath system be made from Lexan. Lexan is nonporous and can readily be sterilized. Frequent sterilization of the Lexan surface will prevent bacteria from reaching a harmful level.
The bending bar also has the potential of bacterial growth. By using surgical steel acupuncture needles, the bending bar is easily sterilized. Because surgical steel needles are inexpensive, it will also be possible to replace needles after multiple tests.

**Parts and Accessories**

- Dell Computer equipped with LabVIEW and MatLAB
- PCI-7330 Motion Controller
- SH68-SH68 Cable
- MID-7604/7602 Stepper Drive
• Danaher Motion Linear Drive
• Danaher Motion Stepper Drive

• Sliding Mechanism

• Fischer Scientific Temperature Regulator
- Removable Bending Bar Piece
- Mounting Baths
- Mightex CMOS Camera
Features:

- Sliding Mechanism controlled through LabVIEW coding
- Transmural stress and strains presented to the user through MATLAB graphs
- Live image from CMOS camera displayed on front panel
- Removable bending bar piece for easy calibration
# Table of Contents:

1.0 Introduction  
   1.1 General Overview  
   1.2 Instruction  

2.0 Maintenance  
   2.1 Electrical  
   2.2 Mechanical  
   2.3 Chemical  

3.0 Technical Description  
   3.1 Hardware subunits  
      3.1.1 Mounting Bath  
      3.1.2 Sliding Mechanism  
      3.1.3 Motor System  
      3.1.4 Image Acquisition  
      3.1.5 Calculation Output Software  
   3.2 Theory Behind Calculations  

4.0 Trouble Shooting  
   4.1 Hardware components  
      4.1.1 Motor  
      4.1.2 Camera  
      4.1.3 Pump and Water Bath  
      4.1.4 Bending Bar  
      4.1.5 Camera mount  
   4.2 Software  
      4.2.1 Motor Control  
      4.2.2 LabVIEW  
      4.2.3 MATLAB  

Page 7  
Page 7  

Page 11  

Page 15  
Page 15  
Page 16  

Page 16  
Page 17  
Page 18  
Page 19  
Page 20  
Page 21  
Page 28  

Page 30  
Page 30  
Page 30  
Page 33  
Page 33  
Page 33  
Page 35  
Page 36  
Page 36  
Page 38  
Page 42
1.0 Introduction

1.1 General Overview

The three-point bending device should be used to measure the flexural properties and transmural strains of tissue composites. The testing is achieved by placing a test specimen against the posts in the inner bath. The tissue will become deformed by a bending bar, which is moved by a stepper motor. During the deformation of the tissue, markers placed on the tissue will be tracked using a Mightex CMOS camera and a LabVIEW program designed specifically for this device. The camera moves as the tissue is deformed to ensure the specimen is always in the camera’s frame of view. The results of the experiment are then calculated in MatLab and outputted to the user.

This device consists of many different components. The mounting baths were designed and fabricated to maintain and control a proper testing environment for accurate and repeatable testing. The inner bath provides an area to place the test specimen. Tissues are laid against stationary posts placed in the inner bath. Figure 1 displays the set-up for the tissue against the stationary posts. Temperature regulation of the entire system is controlled by a Fisher-Scientific Temperature Regulator connected to the outer bath via hose barbs and nylon tubing. Both mounting baths can be seen in figure 2.

![Figure 1. Tissue in Inner Bath](image-url)
The sliding mechanism holds a removable bending bar piece, where the bending bars are placed. The bending bars apply force to the test sample so deformation can be observed. By analyzing the distance between the bending bar and the reference bar during tissue deformation, and comparing the results to data from a calibration procedure, the amount of force applied to the tissue was determined and used to calculate transmural and flexural properties. The piece is removable for easy calibration and storage. The camera is mounted on the device to ensure the camera captures the deformation of the test sample.

**Sliding Mechanism**
The motor system provides motion needed to push the sliding mechanism and aid in the force application of the bending bar into the tissue specimen. The motor system consists of a linear actuator connected to a stepper motor. The stepper motor is connected to a stepper drive, which connects to an NI motion controller in the computer, so the motion can be controlled via a program developed in LabVIEW. The motor system is shown in figure 6 below.
**Figure 6: Motor System Consisting of Stepper Motor and Linear Actuator**

**Image Acquisition**

The Mightex CMOS camera is mounted on the sliding mechanism to enable it to follow the test specimen during deformation. The piece it is mounted in is adjustable to allow for an easy adjustment of the frame of view. The camera is connected to the computer via a USB cable. The images are acquired into a LabVIEW program designed specifically for this device. Figure 7 displays the image acquisition system and figure 8 demonstrates the adjustable field of view for the camera.

![Image Acquisition System](image1.png)

**Figure 7: Image Acquisition**
1.2 Instruction

To start the appropriate software must be opened on the PC. MatLab must be opened prior to testing. The LabVIEW program must then be opened. Ensure the USB camera is properly connected to the computer. Before testing of a specimen, the calibration procedure must be performed by placing weights of known values on the bending bar. To test a specimen, the outer bath should be filled with phosphate buffer saline solution and the Fisher-Scientific Flow Regulator should be turned on. The stepper drive must also be turned on. Place the bending bars on their appropriate spot on the sliding mechanism. Measure the thickness and width of the tissue specimen before starting the test. To begin testing, mark the tissue for the test to be performed and place it in the inner bath against the fixed and free posts. Glue the tissue to the fixed post using superglue or a similar tissue adhesive. Select the desired testing speed for the linear actuator. For both procedures, you will be prompted to identify the two posts and two bars. For the flexural rigidity, you will also be prompted to perform distance calibration by selecting two points in the image that lie on a ruler. You will also be prompted to enter the real world distance between these two points. For the transmural strain procedure, will be prompted to select three points that lie on the outer surface of the tissue’s curvature (this could be near or far to the bending bar, depending on which way the tissue curvature is facing). Begin testing once all prompts have been addressed. All outputs are clearly labeled.

To begin testing using the software, press the run button in the LabVIEW program. Once the run button is pressed, turn to the “Carriage Positioning” tab on the front panel. Use this tab to adjust the position of the motor and the bending bar. Make sure that the bending bar is placed close to the tissue before testing. Figure 9 shows an image of this tab.
After adjusting the position of the motor, go to the “Image Setup” tab. This tab adjusts the image quality. A continuous image obtained from the camera should already be shown in the Camera Display. Use the “Settings” button to adjust the settings for the camera until an optimal image is obtained. Then, press the “Raw” button to process the image. Here you can make sure the markers are being acquired by the camera. In addition, the “Areas of Interest” can also be set such that only specific markers within a specific area on the image are tracked. Figure 10 shows the image setup tab.
Once the image setup is completed, go to the “Testing” tab. This tab performs actual flexural testing of the tissue. The following inputs are required: the number of cycle, the initial speed of the motor, and the desired displacement of the motor. In addition, the user has to input in a directory for where the data will be saved. This can be done by creating a blank text file. After the user has inputted in all the necessary information, the START button can be pressed. Once the button is pressed, the testing will be performed automatically according to the specifications inputted by the user. In addition, there is also a CANCEL button. This button, when pressed, will cancel all the testing and return program to a state where the user can now adjust the motor position and image again. Figure 11 below shows the testing tab. Once the testing is completed, the necessary data will be saved to a text file in the directory designated by the user. In addition, the necessary results will also be outputted by the program and shown in the “Results” tab in the front panel. Figure 12 shows the Results tab.
Figure 11: Testing Tab

Figure 12: Results Tab
2.0 Maintenance

2.1 Electrical

The electrical components of the project are the stepper motor, stepper drive, Mightex CMOS camera, temperature regulator and Dell PC. Frequent testing in the laboratory will require constant evaluation of electrical equipment and wires. A piece by piece maintenance protocol is described below.

The stepper motor consists of five wires attached to a stepper drive. It is important to constantly check the wires for slits or any other damage that may cause harm to the user. The connection to the stepper drive is another thing that must be frequently checked. If one of the five wires comes free from the pin that holds it to the stepper drive, the device will malfunction and one wire will not be grounded. The stepper motor should be placed in a position clear of any water or PBS.

The stepper drive receives input from the motion controller in the PC and sends current to the stepper motor. The SH68-SH68 cable that connects the motion controller to the stepper drive should be checked for proper attachment on both ends as well as damage to the actual cord itself. Dipswitches on the front of the stepper drive must also be checked to make sure that proper current values are being sent to the stepper motor. Similar to the stepper motor, it is essential to keep the stepper drive away from the water or PBS in the mounting baths.

The CMOS camera must be kept dry for proper usage. If any damage occurs to the USB cable connecting the camera to the PC, it can easily be replaced by ordering through Mightex.

The temperature regulator is designed such that all of the electronics are covered by a casing. There should be no issue with the regulator unless the casing is damaged or the power cord has physical damage. Because of this, the casing should periodically be checked for cracks and the wire checked for tears.

The Dell PC should be checked to make sure the motion controller is correctly placed within it. The SH68-SH68 cable should also be checked for proper attachment. PBS and water from the water bath should also be kept a safe distance from the PC.

2.2 Mechanical

The mechanical aspects of the project are the sliding mechanism, the frame and the linear actuator and stepper motor system. Each part of the project has its own unique concerns that must be checked periodically.
The sliding mechanism will frequently travel up and down the frame during the testing. It is important to keep the wheels well greased to make sure that as little friction as possible occurs during testing. It is also important to make sure that the adjustable knob for the camera piece is tightened down before each test run. The grooves in the frame must be frequently cleaned to further prevent friction.

The linear actuator and stepper motor system are coupled together by a custom made piece of aluminum. It is important to make sure that all screws are tied down tightly and that nothing will come loose during testing. The linear actuator must be checked to make sure the four screws holding it to the testing board are also firmly in place.

Chemical

Phosphate buffered saline solution will be used during testing to keep tissues around a pH of 7.4. The PBS solution may cause corrosion of the bending bars and even the Lexan of the inner bath. When the bending bars have corroded to a point where they are no longer usable, replacement bending bars are available and easily swapped in for the old ones.

Because live tissue specimens will be used during testing, sterilization becomes an essential aspect of maintenance. After each test run, bending bars and the inner bath should be sterilized.

3.0 Technical Description

The three point bending device contains multiple subunits. There are the physical components, the software to control the motion of the components and the software to calculate transmural and flexural properties. The following section describes each subunit and how it affects the final device.

3.1 Hardware Subunits

As described previously, the project design consists of subunits, which are all integrated into the complete device.

3.1.1 Mounting Baths

The mounting baths were designed and fabricated to maintain and control a proper testing environment for accurate and repeatable testing. The inner bath provides an area to place the test specimen. Tissues are laid against stationary posts placed in the inner bath. Figure 13 displays the set-up for the tissue against the stationary posts. Temperature regulation of the entire system is controlled by a Fisher-Scientific Temperature Regulator.
connected to the outer bath via hose barbs and nylon tubing. The regulator circulates water throughout the outer bath and maintains the temperature around 37°C. The finished product can be seen in figure 14.

Figure 14: Mounting Baths

Figure 15: Temperature Regulator

Figure 23. Tissue in Inner Bath
3.1.2 Sliding Mechanism

The sliding mechanism is the key component to the force application system and also mounts the camera. The device holds a removable bending bar piece, where the bending bars are placed. The bending bars apply force to the test sample so deformation can be observed. By analyzing the distance between the bending bar and the reference bar during tissue deformation, and comparing the results to data from a calibration procedure, the amount of force applied to the tissue was determined and used to calculate transmural and flexural properties. The piece is removable for easy calibration and storage. The camera is mounted on the device to ensure the camera captures the deformation of the test sample.

![Figure 16: Sliding Mechanism](image)

3.1.3 Motor System

The motor system provides motion needed to push the sliding mechanism and aid in the force application of the bending bar into the tissue specimen. The motor system consists of a linear actuator connected to a stepper motor. The stepper motor is connected to a stepper
drive, which connects to an NI motion controller in the computer, so the motion can be controlled via a program developed in LabVIEW. The motor system is shown in figure 18 below.

![Figure 18: Motor System Consisting of Stepper Motor and Linear Actuator](image)

### 3.1.4 Image Acquisition

The Mightex CMOS camera is mounted on the sliding mechanism to enable it to follow the test specimen during deformation. The piece it is mounted in is adjustable to allow for an easy adjustment of the frame of view. The camera is connected to the computer via a USB cable. The images are acquired into a LabVIEW program designed specifically for this device. Figure 19 displays the image acquisition system and figure 20 demonstrates the adjustable field of view for the camera.

![Figure 19: Image Acquisition](image)
3.1.5 Calculation and Output Software

To join together the physical components with the calculations, LabVIEW and MATLAB were used. The stepper drive connects to the computer which is able to use these two programs to integrate all the components. The calculations begin in LabVIEW with the motor control to position the camera at the correct position, shown below are both the front panel (Figure 21) and block diagram (Figure 22). The force from the carriage movement is used in calculations within MATLAB.
Figure 21. Front panel control showing carriage positioning.

Figure 22. Block diagram of carriage movements. The top portion moves the cart left, the bottom portion moves the cart right.

After the cart position is in the desired position, the camera and image setup needs to be done. There are many different aspects that go into the image setup. The adjustable parameters are the threshold, minimum marker position, maximum marker position, area of interest, creating a bounding box, and various technical aspects within settings. Figure 23 and
Figure 24 below, depict the front panel and block diagram of integrating the physical aspect of the camera to create markers that are used in testing.

Figure 24. The block diagram of image acquisition. The left portion captures the image and sends it over to the right portion which begins to create marker positions.

One of the most technical and important aspects of testing, is the correct positioning and maintenance of marker position throughout testing. This is extremely important because they
are necessary in tracking the tissue deformation and sending the correct position of the deformed tissue through the program. Without this working properly, the results would not be accurate. The user needs to input the number of cycles wanted for testing, the initial speed of the cart being used for the current test as well as the displacement found from the calibration based on the same force being applied. The user also needs to specify a location that the testing will be saved to, which the results tab will open shortly thereafter. Shown below in Figure 25 and Figure 26 are again the front and block diagrams depicting the programming.

Figure 25. Front panel of the testing tab.
Figure 26. Block diagram of the carriage movement, image acquisition, and beginning aspects of the testing. There are many more cases involved that are not shown above.

Once the testing has begun, it will output the marker positions as well as other data to the MATLAB script to compute the calculations of transmural strain. The user needs to define, through inputting, the tissue thickness which will be measured before being placed into the device, as well as the tissue indexes. This verifies that the index wanted and specified, matched the indexes being passed through from the marker positioning portion. The scalar output is also outputted to the user as well as the undeformed and deformed marker positions. It will also open MATLAB and output 8 different graphs, which can be seen in below in Figure 29 and Figure 30.
Figure 27. Front panel of results tab. Only one portion of the outputted data. This only includes the tables. The graphs can be seen in the following section.
Figure 28. MATLAB script within LabVIEW program which calculates the results. This portion of script calls many other MATLAB functions to be used in the program.
Figure 29. Theoretical results of A) undeformed marker position, B) deformed marker position, C) holds the places within the program while calculating, and D) the position of
3.2 Theory Behind the Calculations

The calculations in this program were relatively simple in theory, however, they were difficult to program. The theory behind the physics of these calculations can be seen in Figure 31, the free body diagram of the test.
All of the following equations are based on theory developed by Eric Siros, a PhD. candidate here at the University of Connecticut in the Tissue Mechanics Lab. These equations were all programmed within MATLAB code to be integrated with the LabVIEW marker tracking program to perform all calculations. There are over 60 separate MATLAB functions that include over 10,000 lines of programming.

\[ M = \bar{E} I \Delta \kappa \]  
Equation 1

\[ \bar{E} = \text{instantaneous effective modulus} \]
\[ I = \text{second moment of inertia} \]
\[ \Delta \kappa = \text{change in radius curvature} \]
\[ M = \text{moment that is applied to the tissue specimen} \]

\[ I = \frac{t^3 w}{12} \]  
Equation 2

\[ M = F_x y + F_y x \]  
Equation 3

\[ F_x, F_y = x \text{ and } y \text{ components of reactionary force} \]
\[ X, y = \text{current (x,y) coordinate pair of the point being analyzed.} \]

\[ R = \frac{|AB| |BC| |CA|}{\sqrt{(|AB| + |BC| + |CA|)(|AB| - |BC| + |CA|)(|AB| + |BC| - |CA|)(|CA| - |AB| + |BC|)}} \]  
Equation 4

\[ AB, BC, CA = \text{edges of triangle during testing deformation} \]
4.0 Trouble Shooting

One of the important aspects once all the components are ready for testing, is verifying that the multiple components are working correctly. As with any technical and mechanical machine, there can be glitches and breakdowns that hopefully will not happen, but there is always a potential for unforeseen problems to arise. The following sections go over a few helpful hints in getting the 3-Point Bending Device to work properly.

4.1 Hardware Components

4.1.1 Motor

If the motor is not correctly running, there are a few options that can be looked into. The first check point should be to see if all the physical components are attached firmly and correctly.

![Figure 32. Back view of the correct positioning for the stepper drive.](image)

The stepper motor should be correctly placed into the back of the stepper drive into Axis 3. If it is placed into any other stepper motor location, the programs will not be sending the information to the motor. Verify that all the wire connections are secure and that there are not any exposed wires that could potentially cause a short. The next component to check would be to look at the linear actuator and visually inspect it to make sure that the stage is not pressed firmly against the front or back of this. This is important because if the stage is all the way forward or back, there is no room for movement to occur, which would lead to the assumption that the motor is not working.

Another check would include looking at the front panel of the MID stepper drive. If the LED lights are on, as seen in Figure 32 below, should refer to the software trouble shooting section further in the operator’s manual.
The front panel of the stepper drive should look like Figure 33, without any error lights as well as having the correct dip switches in the correct positions. This can be seen in Figure 33, but the dip switches for Axis 3 should read as follows: down positions-1,4,5,6,9 and 10, up position- 2,3,7, and 8. Figures 34 and 35 give brief examples of common errors and tips.
Figure 34. Off position for stepper drive. Always turn off once done with testing to maintain device.

Figure 35. Always verify that the power of the stepper drive is on (green +5V LED light) before testing. This is a common error since it is not involved with the LabVIEW program.

4.1.2 Camera

The camera is a crucial aspect of the device and needs to be working at its highest potential. If the image is not extremely clear, there are multiple things that can be done. The first should be that the cables are all connected to the correct ports and firmly attached. Once this is confirmed, the user should be able to open the settings tab on image setup within the LabVIEW program, opening up ScopeTek DCM 301 Properties, seen below in Figures 37 and 38.
Figure 37 can be adjusted through the various parameters but the ones that should be verified that the camera mode is continuous, the exposure center is set to 10 ms as well as the exposure time set to 5 ms. These can all be found on the first tab under the properties. Adjusting the parameters of the frames per second might also help clarify the image by sending slower refresh rates. This can be done as seen in Figure 38.
4.1.3 Pump and water bath

The pump and water bath should be fully functioning on its own, however this might not always happen. The first check should be that the machine is on and plugged into a power source, this can often be overlooked because everything else is controlled through LabVIEW. The next step should be to verify that all the tubes are correctly fastened to the inlets on both sides of the bath. If these steps do not fix the problem, refer to the owner’s manual and specifications for this bath.

4.1.4 Bending bar

Verify that the t-piece is within the holder. The bending bars should be held firmly in place by the set screws which are not visible. They appear as drilled holes into the side of the holding piece and can be set with an extremely small screw driver. These need to be secured.
and firmly in place for the testing to be accurate. It should also be verified that the t-piece is
being held in the correct place and accurately placed snug into the holding piece.

4.1.5 Camera mount

If the camera is still not receiving clear images, the final steps to adjust the picture can
be taken. This is done by adjusting the camera mount. Figure 39 shows the camera mount at a 5° angle. This can be adjusted by turning both of the black knobs. Loosening them allows the
user to choose the angle which the device will run at. There is a 10° total adjustment allowance
can help the user find the best angle for viewing the testing while running. Figure 40 shows the
camera at the +5° angle, with the knob positioned fully at the top of the groove.

Figure 39. -5° camera mount position.
4.2 Software

4.2.1 Motor Control

The motor control program should be looked at if the steps have been taken in the hardware troubleshooting and the motor is still not working. The first step is to look at the Measurement and Automation program. The following tabs need to be selected to be able to adjust the parameters: devices and interfaces, NI motion devices, PCI-7330, default 7330 settings, axis 3. Seen below in Figure 41, these steps allow the user to again initialize the motor within the stepper drive. This should be done each time the stepper drive is disconnected from the computer. Toggling through the various tabs, the user will be able to modify the direct motion as well as the other various parameters.
Figure 41. Measurement and automation Axis 3 configuration.

Once this is done, the next step would be to choose the 1-D interactive tab. Pictured in Figure 42, 1-D should be chosen because the linear actuator is only moving in a 1-D manner and does not need a y coordinate. The user should go through the following checklist while troubleshooting the motion: verify axis 3 is selected, open loop stepper mode, operation mode is selected as relative position, and apply all these parameters. Once this is done, the start button on the top left should be pressed to verify that the motor is now correctly running.
4.2.2 LabVIEW

This LabVIEW program is very complex and has many different things to look at. The user would need to become an expert to debug the program, however, the following can be looked at briefly to help the program run.

When the program is running, is the user is not able to detect any markers, this is likely because the raw image tab is not depressed. When this tab appears gray, it will only show what the camera is exactly looking at as seen in Figure 43. When the tab is depressed, Figure 44, this shows the user what the image acquisition program is looking at. This should be able to show the marker positions by the small red boxes which can be seen. The marker number is then displayed. If this does not fix the problem, the next step should be to verify that the light source is accurately giving enough light. Figures 43 and 44 show images lacking a strong lighting source and performed just with overhead lights on. Figure 45, shows the image displayed with two lamps directly exactly towards the tissue test. This allows the markers to be seen easily and will provide the most accurate calculations. Another frequent error in LabVIEW can be seen in Figure 46 below. This message will appear after the program runs through and will then

Figure 42. Measurement and automation interactive tab.
unexpectedly crash. This might occur often and shutting down both the LabVIEW program and the MATLAB program should be done. If this problem still continues, then the entire computer should be restarted and this should fix the problem.

Figure 43. Image setup not being able to detect any markers.
Figure 46. Image setup able to find markers incorrectly.
Figure 47. Image setup with correct lighting.

LabVIEW 8.5 Development System has encountered a problem and needs to close. We are sorry for the inconvenience.

If you were in the middle of something, the information you were working on might be lost.

Please tell Microsoft about this problem.
We have created an error report that you can send to us. We will treat this report as confidential and anonymous.

To see what data this error report contains, click here.

Figure 48. LabVIEW error message.
4.2.3 MATLAB

The most common MATLAB error occurs with the integration of the MATLAB code with LabVIEW. The MATLAB script in LabVIEW version 8.5 cannot accurately open the MATLAB program. The user needs to manually do this before testing. Error 1047, seen in Figure 49, is a common error that appears when LabVIEW is opened and run before MATLAB is opened. Other error messages associated with this are error numbers 1048 and 1050, these all deal with the MATLAB script compatibility issues. To fix this problem, simply open up MATLAB 2009b program before opening the LabVIEW program to run the test. This should alleviate this problem but if it persists, the computer should be restarted.

![LabVIEW error message](image)

Figure 49. LabVIEW error message resulted from MATLAB script.