Procedure for mounting a sample within the apparatus

Materials required:

Test apparatus

2 Newport linear actuators and their respective Conex controllers

Conex AC/DC power adapter and supply cable

Conex DC jump cable (about 5 inches in length)

2 USB to USB-mini A cords

Windows computer with CONEX-CC GUI installed

1. **Setup**
    a. Ensure that all actuators are securely mounted within the apparatus.
    b. Ensure that actuators have been preset so as not to cause collision between the load cell and the holder.
    c. Insert USB-A cords into the port on each controller and connect the other end to the computer with the CONEX-CC controller program installed.
    d. Plug the CONEX power supply into the wall. Insert the other end of the cord to the DC IN power port on one of the two controllers. Use the jumper cord supplied by Conex and place it between the DC OUT port on the controller connected to the power supply and the DC IN port on the second controller.
2. **Initialization**
    a. Open the CONEX-CC Controller program. Ensure that both actuators are recognized by the computer and launch the applet.

b. Both controllers should be in a NOT REFERENCED state with an orange led displaying. Under the “Diagnostics” tab, enter the command “1PW1” and send to the X controller. If the controller for the compressive actuator is blinking red, the X controller moves the compressive actuator, and the Y controller moves the shear actuator. If the controller for the shear actuator is blinking red, the reverse is true. Starting with the X controller, send the command “1HT1” in the diagnostics tab. Once successfully completed, send the command “1PW0” to the X controller and repeat the same three commands in order for the Y controller. **This eliminates the risk of the controller reaching apparatus limits before its end of run during its initialization and subsequent potential damage to the load cell**. Unexpected behavior from the controllers is explained in a straightforward manner in the documentation for Conex-CC controllers and may be found at http://search.newport.com/?x2=sku&q2=CONEX-LTA-HL under the “Literature and Downloads” Tab.

c. After setting the home targets as directed in step 2b, initialize both controllers by clicking “Initialize on their respective tabs. Using the “Move Relative” option, incrementally move the compressive controller, and ensure that the minimum gap between the holder and the load cell (determined in this case by using a thin “feeler gauge”) is set as the maximum ‘end-of-run’ for the compressive controller.

3. **Sample Mounting**

a. Using the move relative command, move the compressive actuator away from the load cell until ample room is available for the mounting operation.

b. Power up the boxes receiving data from the load cell and initialize the LABVIEW GUI (as found in the start menu) for further data collection and processing.

c. Use cyanoacrylate glue to adhere the cartilage sample to the titanium mount. Once adequate cure time has been allotted (~1 minute) apply cyanoacrylate glue to the other side of the cartilage sample, and insert the mount into the bath-style titanium component as rapidly as is possible without the risk of compromise of the equipment set-up.

d. Once the mount has been inserted, use the compressive actuator to bring the holder attached to the load cell into complete contact with the cartilage sample so as to allow for proper curing of the cyanoacrylate glue between the cartilage and the second metal mount.

e. Once the sample has been glued and mounted and the glue allowed to cure, use a flat, very sharp blade to cut off the excess cartilage above the plane of the top surface of the two titanium arms (which have been machined to be coplanar upon apparatus setup). This allows for a minimum of wasted images near the surface of the sample. Approach with the blade from the side at a slight angle so as not to accidentally gouge the center of the sample between the mounts.

4. Imaging procedure

a. Insert the apparatus into the two-photon microscope and ensure that all actuators are still connected and have not moved. **Secure the apparatus and sample inside the microscope prior to imaging or applying displacements.** As a precautionary measure, reduce the maximum velocity of the actuators to 0.1mm/s instead of the default 1 mm/s speed. Make sure that the computer controlling the actuators will not

turn off or sleep if idle and that it has sufficient battery to remain on for the duration of the data collection.

b. The LABVIEW interface displaying the load cell data should indicate a steady but not overly high load (both in forces and moments). Zero the force and moment readout by toggling the bias button in the LABVIEW display prior to applying any compressive displacement to the cartilage sample.

c. After all prior steps have been completed, the sample is ready for imaging and displacements. **DO NOT APPLY DISPLACEMENTS OR OTHERWISE MOVE THE SAMPLE DURING ACTIVE IMAGING OF A STACK AS THIS RISKS DAMAGE TO THE MICROSCOPE AND WILL INVALIDATE THE IMAGE STACK.**

d. After each image stack is complete and processed, ensure that the LABVIEW GUI is set to save to a new file and begin data collection for later review. After data collection has begun (triple check this as LABVIEW is fickle), is it safe to apply the displacement.

e. Apply the desired displacement to the sample with the actuators. The loads on the sample (both forces and moments) will experience an initial spike followed by an exponential decay toward an approximately steady state, as indicated by the LABVIEW readout. Image stacks take on the order of 2 minutes to collect and thus if relaxation is slow relative to this time scale, images will be valid.

f. Once the image stack has been completed and saved, repeat steps 11 and 12 as necessary to acquire the desired data for comparison.

5. **Conversion using Leica and IMARIS**

a. Ensure that each image stack is saved as a separate experiment in the Leica Software. This can be done manually after all images have been taken by using the Leica AF Lite software to open the experiment, creating a new experiment for each image stack, and then copying the image stacks individually as needed.
b. Use IMARIS 64 to crop the image stacks to the area of interest. If this step is not performed, later analysis of the image files will be restricted to a smaller viewing window than desired, as the maximum array size allowed by the fast iterative DVC algorithm is significantly smaller than the resolution of the image stacks as taken from the microscope. Further cropping may need to be performed in MATLAB after the arrays have been created. A script that creates two cropped arrays for use in the DVC algorithm is included in Appendix A.
c. After imaging has been completed, use IMARIS 64 to compile the images into MATLAB-compatible files (.mat). This is done by running the GetIntensityCh.m script in MATLAB. Note: An IMARIS "Xtension" called ImarisICEConnector is required to run the script. It can be downloaded from http://www.scs2.net/next/index.php?id=110.
d. When IMARIS opens, select the image stack file to be converted.
e. This yields an output in the MATLAB workbench called 'stacko'. Save this variable as a .mat file by right-clicking on it and selecting "Save As…"
f. After all image stacks have been processed as detailed in steps 16 through 19, save the converted arrays to a USB drive or otherwise make it possible to load them onto the computer running the fast-iterative DVC algorithm.