SOP – BioTester 5000 (CellScale)

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Equipment Overview

Biaxial testing is critical for understanding the mechanical properties of biomaterials due to their directionally-oriented microstructures. The instrument provides insight to the response of cells to mechanical stimulation. Displacement and force control, cyclic testing, creep, preloads, and non equi-biaxial loading can be set up in the software. During testing, the software provides continuous feedback to the user through real-time images and data graphing. The image analysis software allows users to review images, track points on the surface of the sample, and quantify local strain fields that can be converted to a video.

Key Features

- A temperature-controlled media bath to maintain samples.
- Several attachment options for gripping a wide range of specimen sizes and properties.
- High resolution imaging for non-contact strain measurement.
- Fully featured user interface software for simple, cyclic, relaxation, and multi-modal testing with real-time feedback.

Force Capacity	23 N
Available Load Cells	1.5 N (others options available with upgrade)
Max Grip Separation	80 mm
Max Velocity	20 mm/s
Max Cycle Frequency	2 Hz
Max Data Rate	100 Hz
Max Image Rate	15 Hz

Notes before use:

- Many BioRakes (for gripping samples) are available for mounting specimens (smallest is 3 mm).
- Any test parameters can be saved as a template for future use.
- Clean all BioTester accessories that will be used with 70% ethanol.

Supplies and Reagents

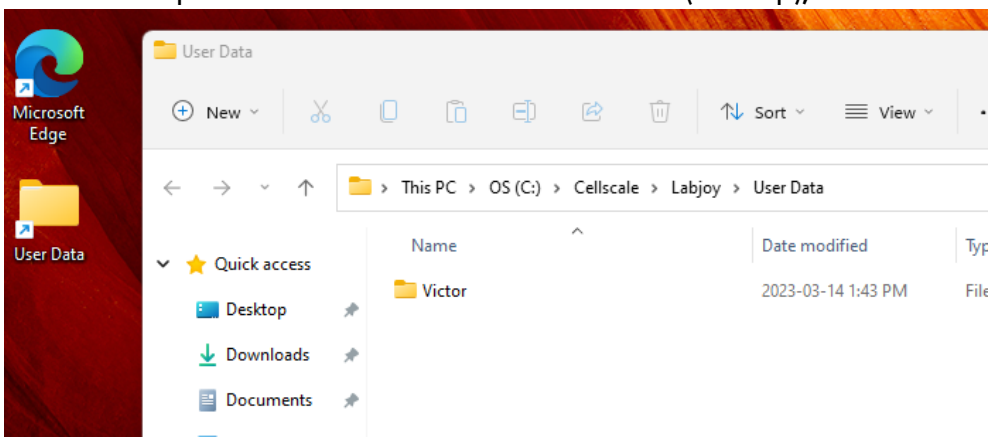
Provided by CRAFT:

- BioRake mounting accessories for attaching samples.
- Stainless steel specimen clamps.
- Disinfectant: 70% ethanol or PREempt RTU (accelerated hydrogen peroxide).
- Kimwipes, and paper towel.
- PPE (lab coat and safety glasses).

Method

Initial Setup:

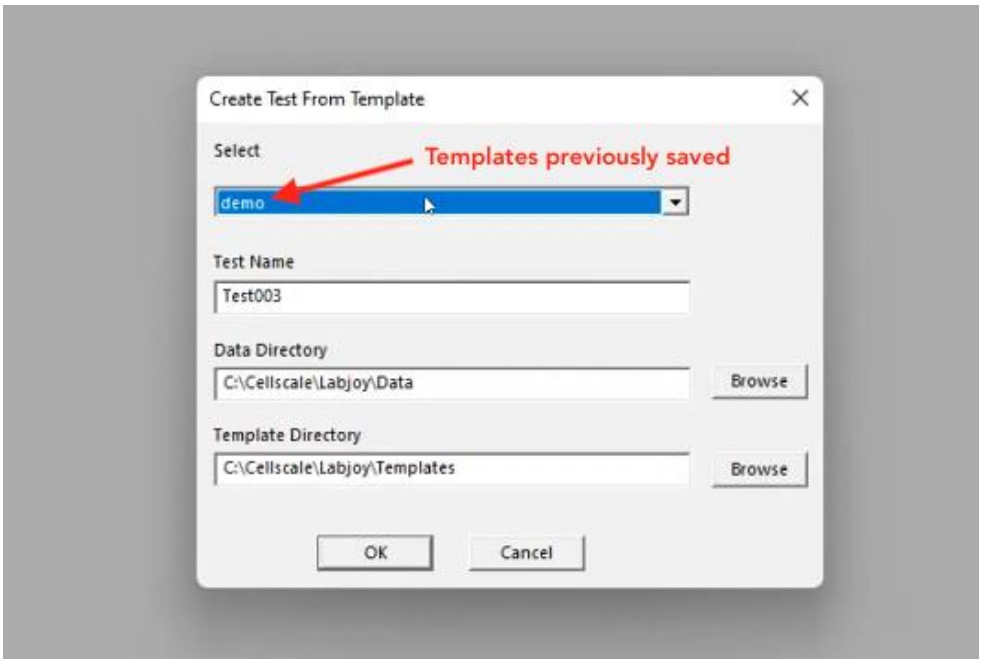
1. Log into LMACS to activate the instrument.
2. Create a personal folder in the **User Data** folder (desktop), if there isn't one already.



3. Ensure the BioTester 5000 is turned on. The instrument has the following:

   - **Main** switch = turns on the BioTester and lights.
   - **Heater** switch = turns on the heating bath underneath the stage for warming up fluids based on the temperature set in the software.
   - **Rod** = raises/lowers the stage of the media bath and sample.
     - **Lift up** = release
     - **Pull** = raise stage, **push** = lower stage

4. Open the software (LabJoy). Click **File** → **Collect New**. A window will appear:

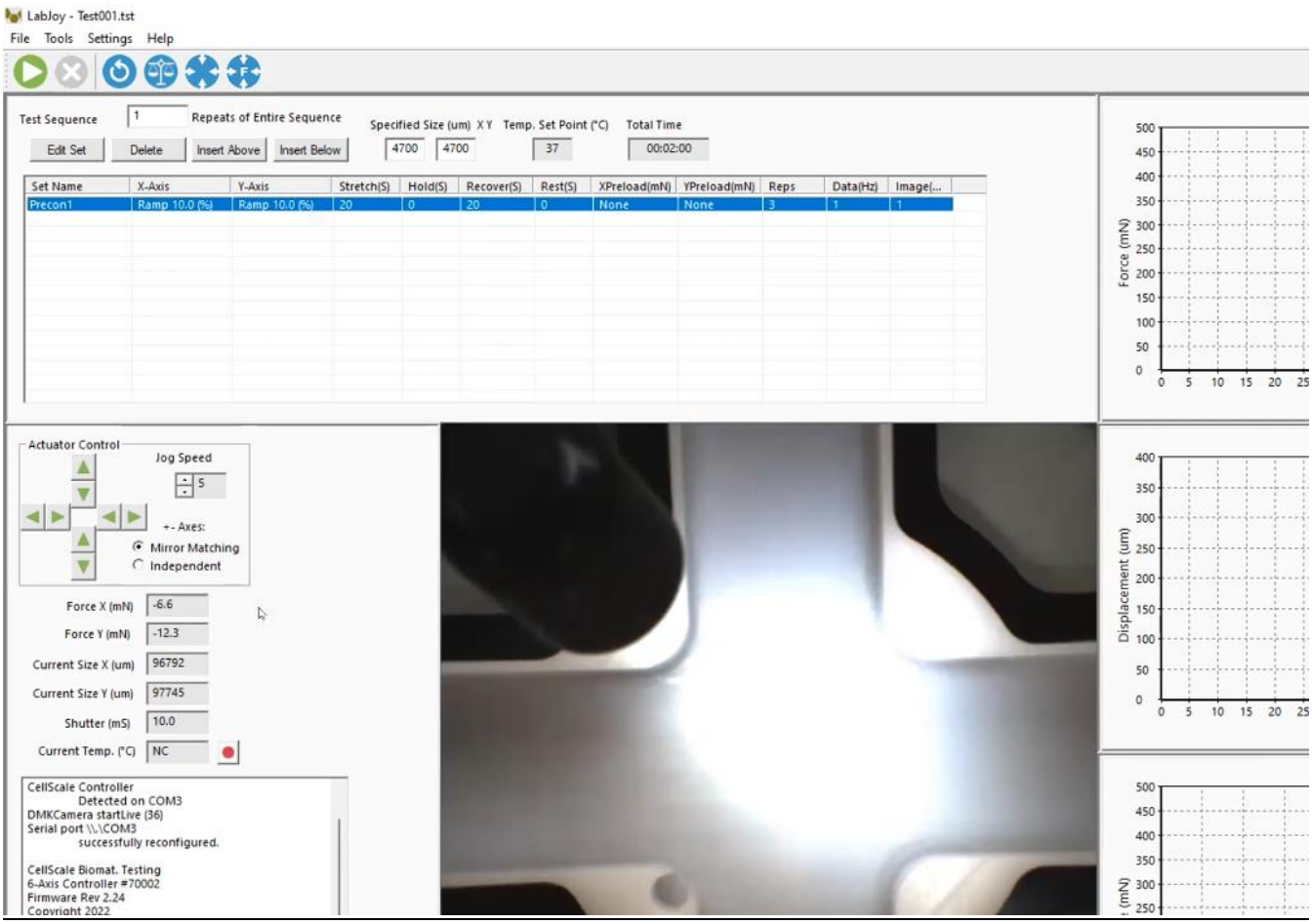


Test Name – You can use the default or rename the test. Each time you start a new test, the default name will have an increased increment number at end of the name (ie. Test003, Test004, etc).

Data Directory – this is where the data will be saved (user folder, USB/external drive).

Template Directory – this is where templates (ie. demo) can be saved and appear in the drop down menu.

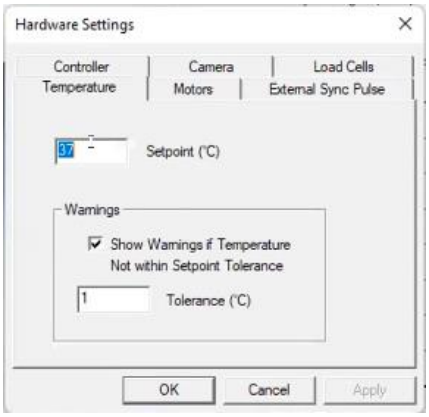
5. Fill in the details, and click **OK**.



Insert Above/Below = to add more experiments

Current Temp. (°C) = represents the temperature of the media bath. To set the temperature, click: **Settings** (top menu) → **Hardware** → **Temperature** tab.

Note: The maximum temperature is 40 ℃. It takes ~1h 15 min to reach the set temperature.

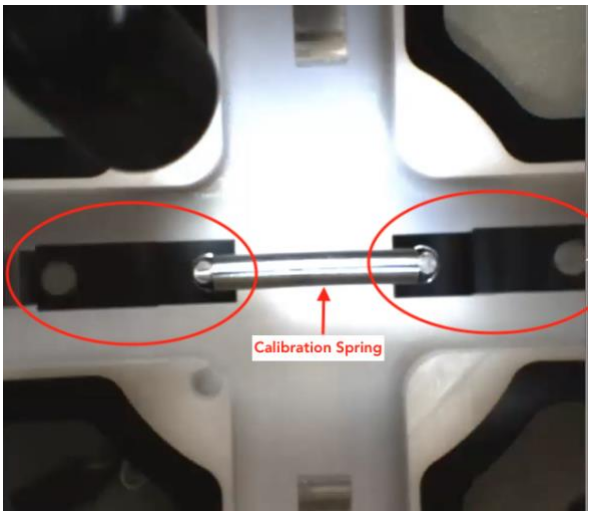


Optional: Zeroing Load Cells

** This is a calibration check using the 1.5N spring provided at CRAFT, and generally done every few months or once a year. The process accommodates for drift from repeated use and should be performed whenever there's physical movement of the actuators. This will ensure force measurements are accurate.*

1. Place the **calibration brackets** onto the actuators on the X-axis. Zero the load cells by clicking **Tools → Zero Load Cells**.

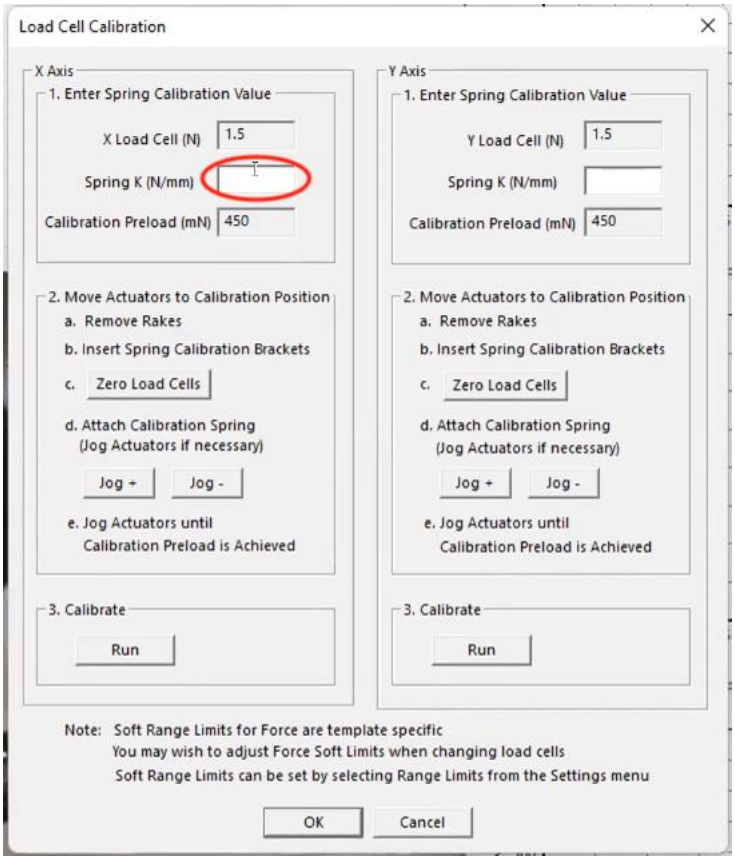
2. Align the actuators using the software (**Actuator Control**) until there is sufficient space to add the **calibration spring** between them:



*Note: the movement speed of actuators can be controlled by adjusting the **Jog Speed** in the software.*

3. Change the *jog speed* to **5** or lower, and gently reach 450 mN.

4. Select **Tools → Advanced → Load Cell Calibration**. Enter the **Spring K value (N/mm) →** 0.02236 (on the box for the 1.5N calibration spring). Click **Run**:



*Note: If the calibration brackets move a lot in this step, it means the spring isn't level and the actuators need to be adjusted (refer to procedure "**Perform Vertical Gooseneck Alignment**" in Appendix C of the User Manual).*

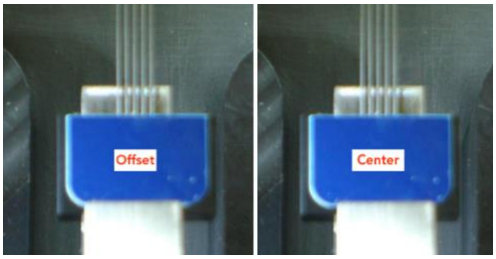
5. *The value will show up on the bottom left screen (ie. A = 1.0127), which should fall between **0.97 – 1.03**. Click **OK**.*

6. Repeat **Steps 1-5** by placing the brackets in the Y-axis.

7. Put away the calibration brackets.

<u>Gooseneck Alignment</u>

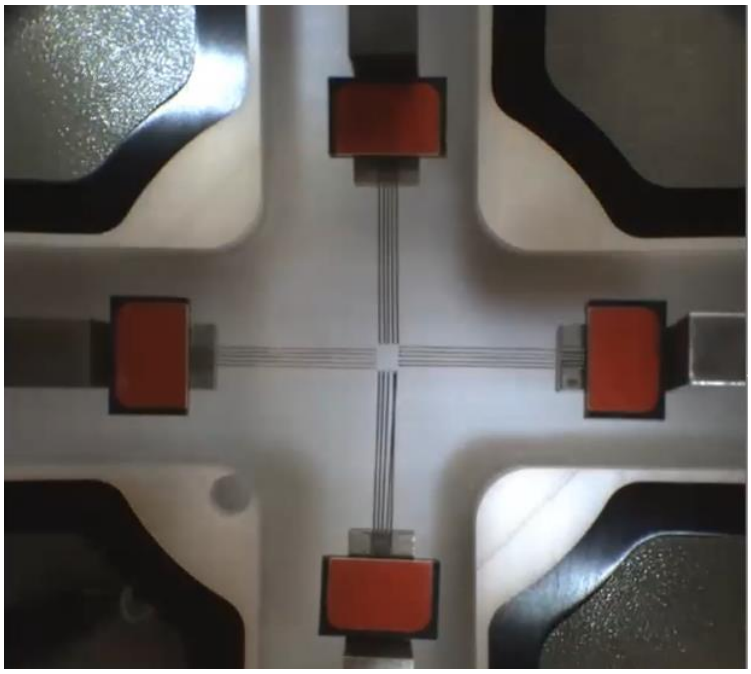
1. Obtain a suitable set of **BioRakes** for your sample and place them in the centre of the 4 actuators.



Note: if gooseneck is out of alignment, seek a CRAFT staff member.

2. Adjust the goosenecks *axially* by clicking the **Independent** radio button from the **Actuator Control**. Increase the jog speed (don't go above 7) and position them to form a square.

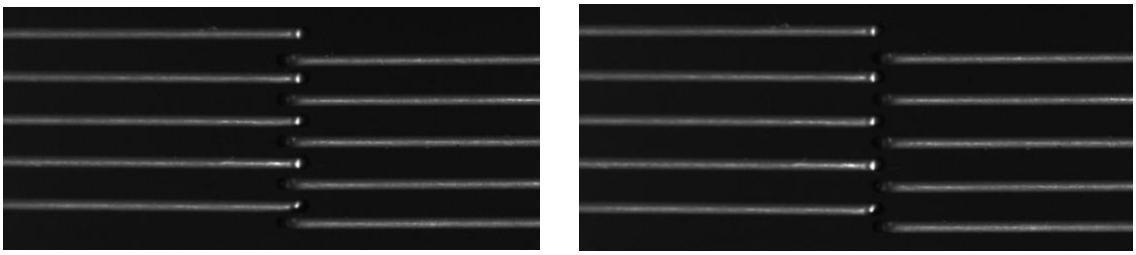
   Note: High jog speeds will cause too much acceleration and inertia on the load cell and affect its accuracy.



3. Adjust the camera position using the knobs on the camera unit, and fine tune the image focus using the lowest ring on the lens.

Note: The camera alignment should be parallel to the rake alignment, and the BioRake tips should be in the middle of the image.

4. Click **Tools** → **Advanced** → **Centre Position Calibration** → **Yes**. Click the **Reset** button (or click Tools → Reset Actuators).
5. Click **Tools** → **Advanced** → **Move to Centre** → **Yes**.
6. Take off the BioRakes for Y-axis set. Set the **Jog Speed** to 5, and **Mirror Matching** in the **Actuator Control**. Move them towards each other and ensure they overlap:



Note: too much overlap (left), correct position (right).

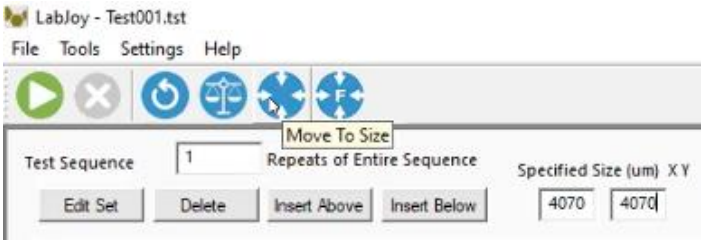
7. Remove the BioRake set, and place the Y-axis set on. Repeat the previous step.

8. When everything is aligned. Click **Tools** → **Advanced** → **Centre Position Calibration** → **Yes**. Reset the actuators by clicking **Tools** → **Reset Actuators**.

9. Based on the sample, change the **Specified Size (um)** for **X** and **Y**.

Note: Input value = sum of all tile distances and tile diameters.

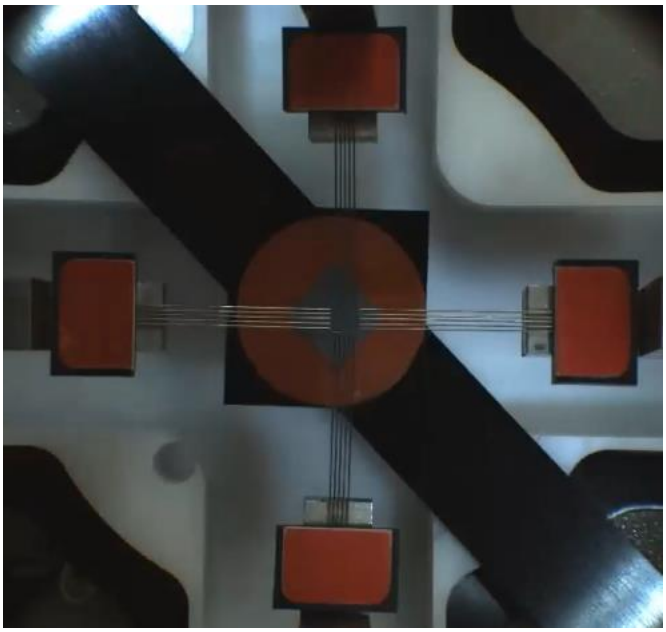
10. Click **Move to Size:**



11. Place the 4 BioRakes onto the actuators and align them to the centre.

Sample Setup:

1. Wipe the orange silicon material with 70% ethanol, and place it onto the **mounting bridge** above the media bath. Gently place the specimen onto the silicon material:



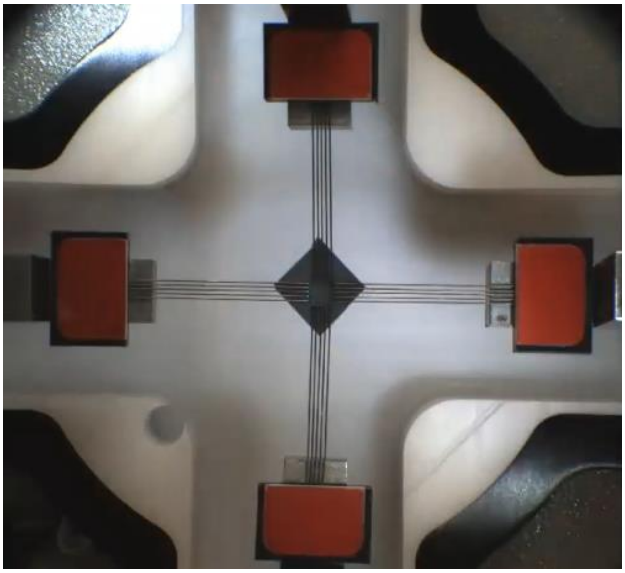
Note: for setup of other types of specimen, refer to the user manual.

2. Lift and pull the **rod** of the BioTester until the BioRakes are close to the sample. Use forceps to centre the sample if necessary.

3. Raise the stage again until the BioRakes attach to the sample and rise *slightly*.

4. Use the **press block accessory** (or fingers) to push down on the sample.

5. Lower the stage slowly, and remove the mounting bridge.

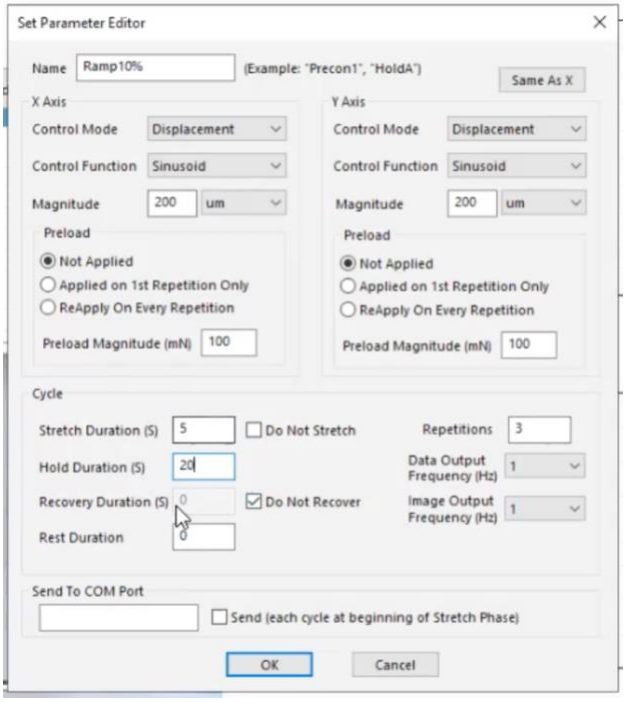


6. Optional: raise the stage to submerge the sample in media if needed. Generally, biological materials are in a saline solution heated to the organism's body temperature.

Experiment Setup:

1. Click on **File → Collect New** to start a new test.
2. Set or modify a protocol by clicking **Edit Set.**
3. Refer to the manual and define the experiment setup as desired. Click **Ok.**

Below is an example test of stretching a sample 200 um for 5 seconds, holding it, and repeating it 3 times (600 um total stretch):

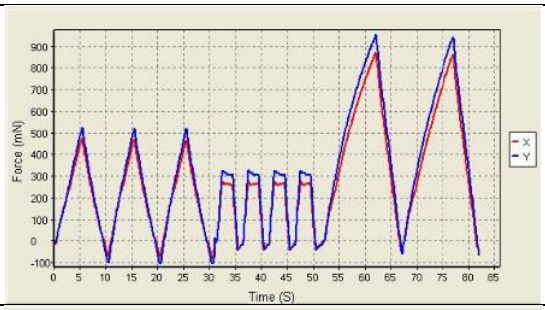
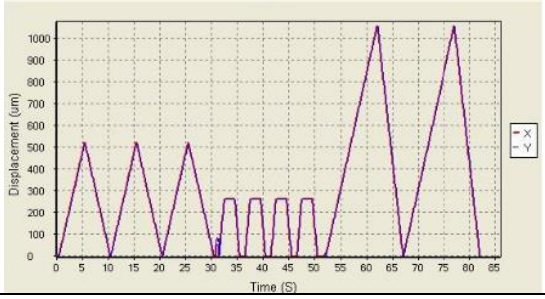


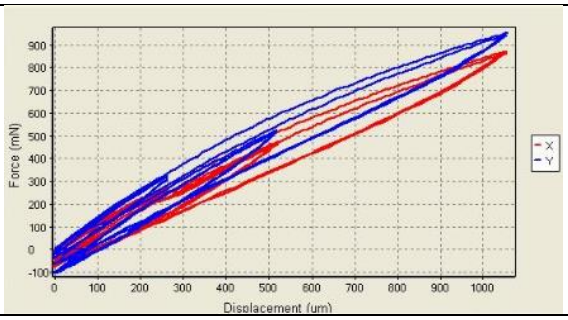
Tip: to find the point where a sample snaps, apply a large stretch magnitude (Ie. 72000 um) and a long duration (ie. 120s).

Test Parameter	Description
Control Mode	Biaxial tests are typically done with displacement control; however, you can select these control modes for specific testing objectives: To obtain uniaxial properties for a biaxially mounted specimen, specify displacement control in one axis and **force control** with zero **load magnitude** in the other axis. For creep testing, use a preload with a long **hold duration**. For relaxation testing, use displacement control with a long **hold duration**.
Control Function	True Strain (**displacement control** mode only), Sinusoidal, or Ramp is for tests performed in **displacement** or **force control** mode.
Stretch Magnitude (Load Magnitude when in **Force Control** mode)	A sound approach is to begin with a small magnitude and iteratively move up to larger magnitudes. If using **displacement control** mode, you can specify the displacement in **µm** or %. For example, the displacement on a 5000 µm (x-axis) x 5000 µm (y-axis) specimen can be expressed as either 500 µm or 10%. If using **force control** mode, force is in **mN**.
Preload	If a sample is originally in a compressed state, the sample size can be adjusted after a preload adjustment. Strain calculations are

	based on the sample size after the last preload adjustment. If a material has a suggested preload value, set it here. Otherwise, zero is a good initial choice.
Preload Magnitude	Depends on the sample. While Preload can be set at 0, typically you set the preload magnitude 0–10% of the peak load you expect to achieve.
Stretch Duration	Defines the time needed to reach a specified **Magnitude**.
Hold Duration	Defines the time for holding the stretch.
Recovery Duration	Defines the time needed to go back to the original un-stretched position. Typically set to the same value as the **Stretch Duration**.
Rest Duration	Typically set to 0. A non-zero value is often used to mimic *in vivo* conditions or for specialized tests.
Repetitions	Apply enough until the force deformation curves from one repetition to the next.
Data Output Frequency	Typically set to the same frequency as the **image output frequency**.
Image Output Frequency	Entering the value 5, would capture 5 image/data points every second. Typically, we set: 1Hz if cycles >5 seconds 10 Hz if cycles <5 seconds

4. Press **Start** on the toolbar when ready. To stop a test prematurely, click . Below are the types of graphs that will be generated:

Force Vs. Time This graph shows how X and Y forces are changing with time. Peak loads per cycle differences in load between the X and Y axes, and force relaxation are seen in this graph. Force is proportional to nominal (engineering) stress.	
Displacement Vs. Time This shows how rakes are moving with time. The phases of the test sequence and size adjustments due to preloading are apparent in this graph. Displacement is proportional to nominal (engineering) strain.	

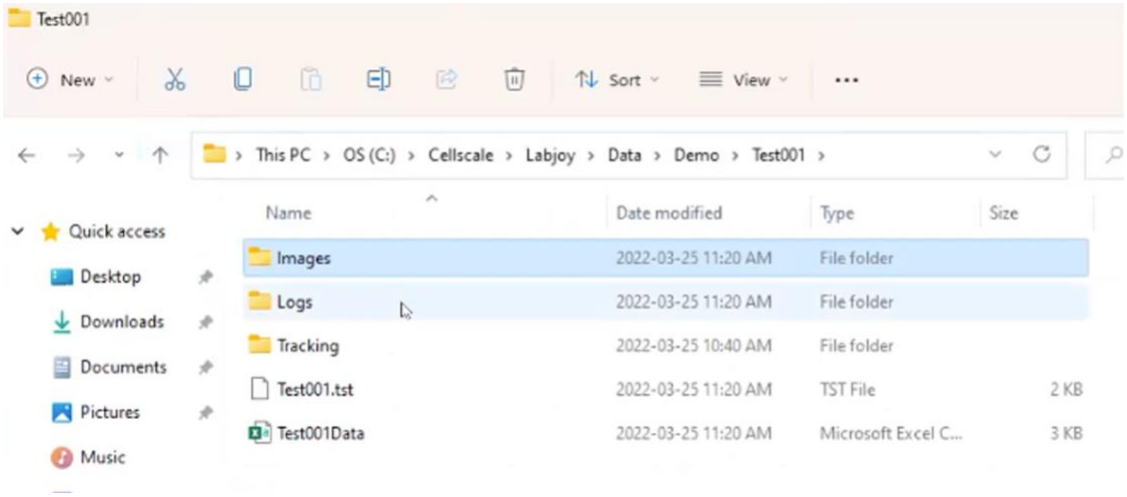
Force Vs. Displacement This graph shows a qualitative representation of material behaviour. Viscoelastic effects (like hysteresis) and material response to different loading phases are apparent in this graph. This is proportional to a nominal (engineering) stress-strain graph.	

*Note: Red is the **X** data, blue is **Y** data.*

5. When the experiment run is finished, the settings and controls will be grayed out. A window with data saving options will appear, which will include the option of saving the experiment template for future use (or deleting previous templates).

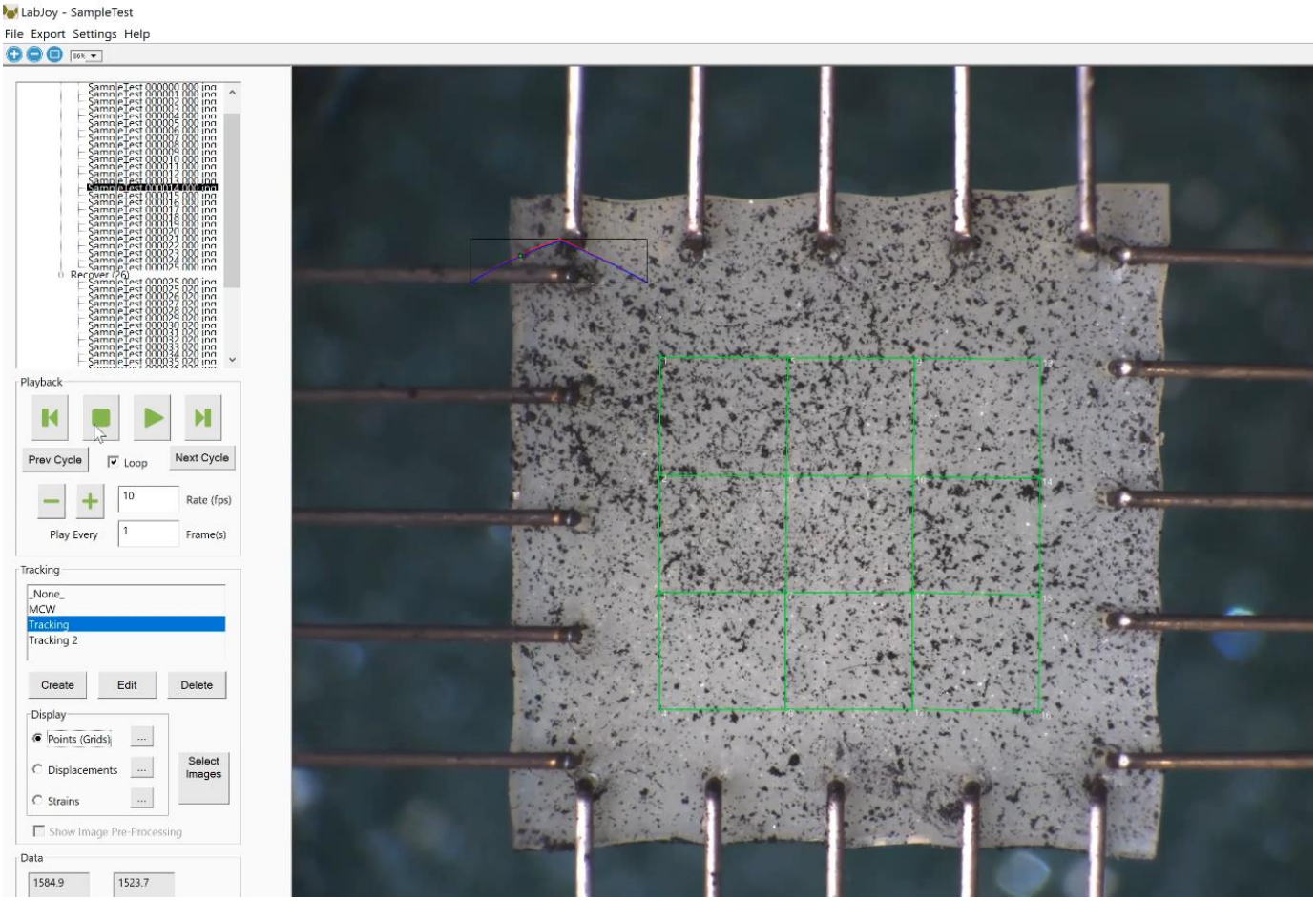
Export & Saving:

1. All data is saved in **C: Drive**, in the forms of the following:
    a. **Images** (live view of sample)
    b. **Logs** (used for tech support)
    c. **Tracking** (for DIC)
    d. **TST** file (contains protocol, settings, data for the test)
    e. **CSV** file (raw data that includes, time, force, and displacement values)

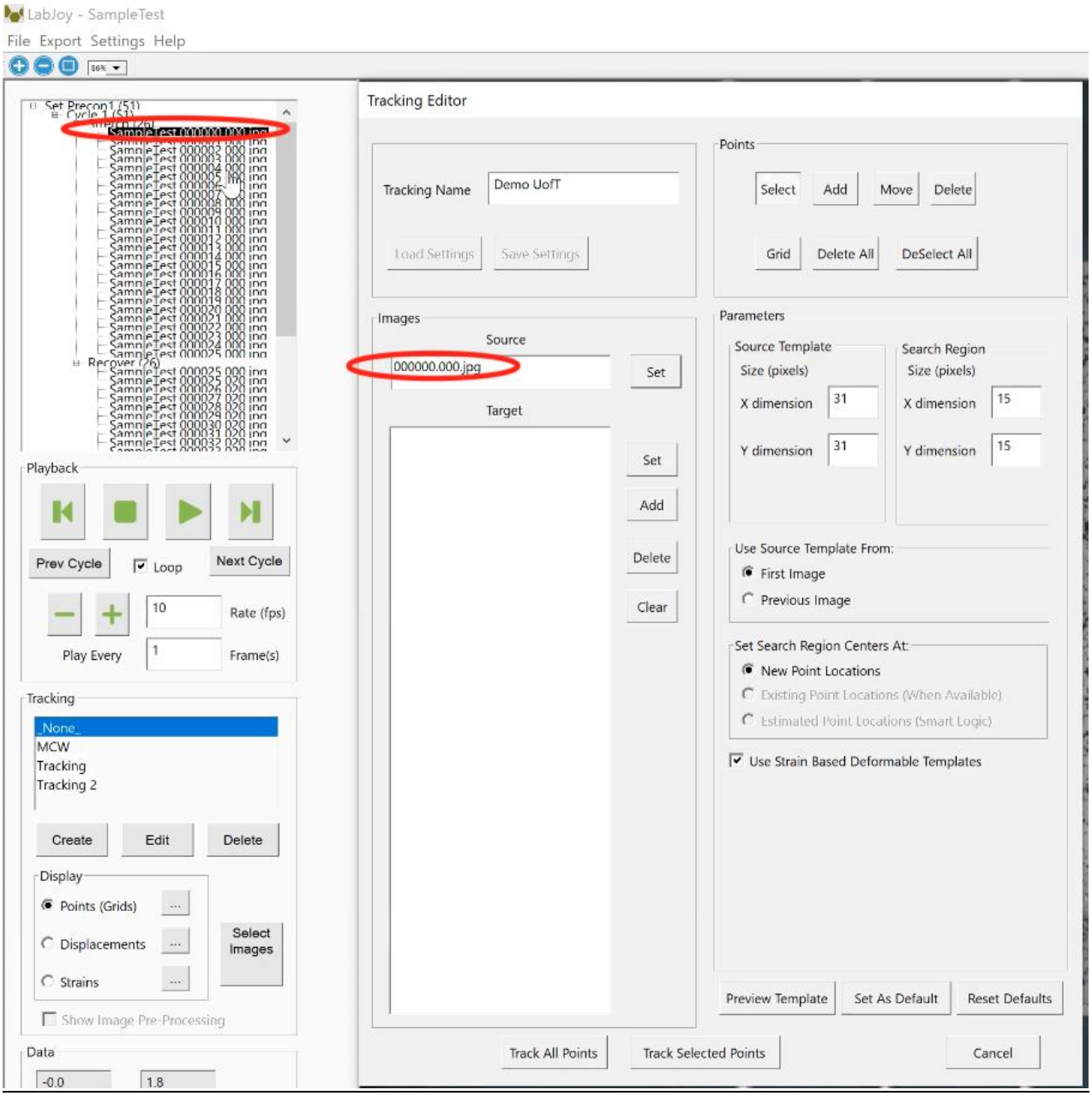


Optional - Post-processing:

1. Click **File → Analyze And Review Images.** Select the desired **TST** file.



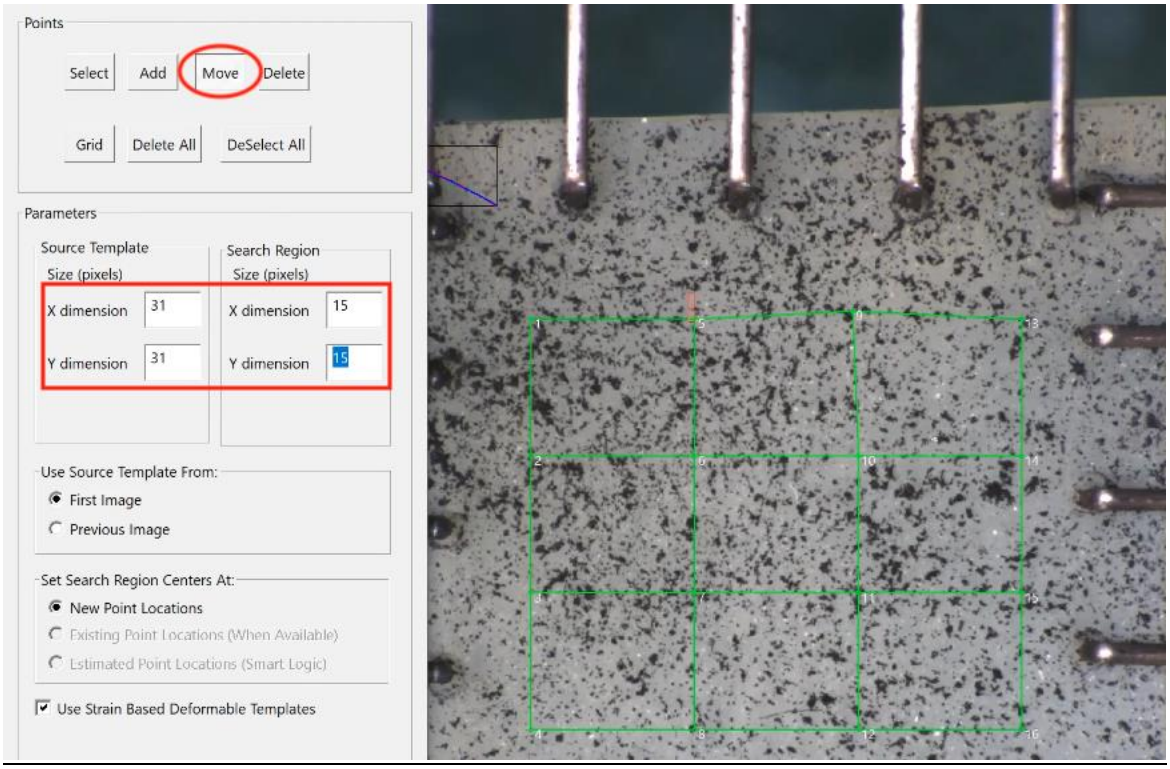
2. In the **Tracking** section, click **_None_** → **Create** to open the Tracking Editor.



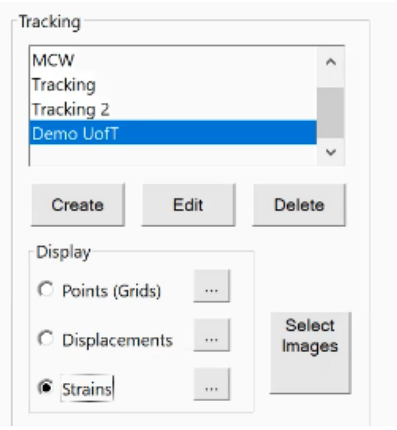
3. Set the **Source**, and select images of interest (ie. cycle) in the **Target** section, and click **Add**.

4. Click **Select** and draw a region of interest using the mouse, click **Grid** (Points section). Define the grid (ie. 4 x 4).

5. The points on the grid can be moved by clicking the **Move** button. In addition, the image dimensions (X/Y) can be modified in the **Parameters** sections. Once satisfied, click **Track All Points**.



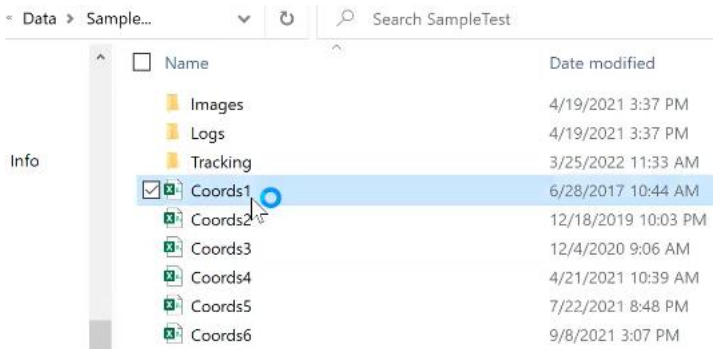
6. When the processing is complete, it will appear under the **Tracking** section (ie. Demo UofT) where displacement and strain data can be assessed.



7. *Optional (to save a csv file for the new tracking):* click **Export** → **Data Points**. Specify a save location for the csv file to be created.

Note: ***Export*** → ***Movie*** *will compile tracking points to create a video.*

8. In the file folder, specific coordinate csv files will be present.



Cleanup:

1. Remove the sample holder (ie. BioRakes, grippers) and sample from the instrument.
2. Clean all tools and accessories used for the experiment setup.
3. Close the software, and shutdown the computer.
4. Wipe the work station with RTU or 70% ethanol.
5. Log out of LMACS.

Troubleshooting:

1. Restart the software if it is not working.
2. Refer there is an error about *actuator range limits*, refer to the User Manual section "***Perform Transverse and Axial Gooseneck Alignment***".
3. Absorb minor spills with Kimwipes (or paper towel), followed by a secondary wipe using 70% ethanol. Contaminated wipes are to be disposed into the yellow biological waste container. If a major spill occurs, refer to the **Spill Kit** underneath the lab sink.
4. If issues cannot be resolved, contact a CRAFT staff member.