

## Assembly and Usage Guide

### Overview

The tensile testing device described here enables simultaneous application and measurement of forces applied to embryonic epithelia *in vivo*. Below, we describe in detail the assembly instructions for building the device, how it has been utilized to perform mechanical analyses of avian endodermal and ectodermal epithelia, and how the provided code can be utilized for subsequent data analyses.

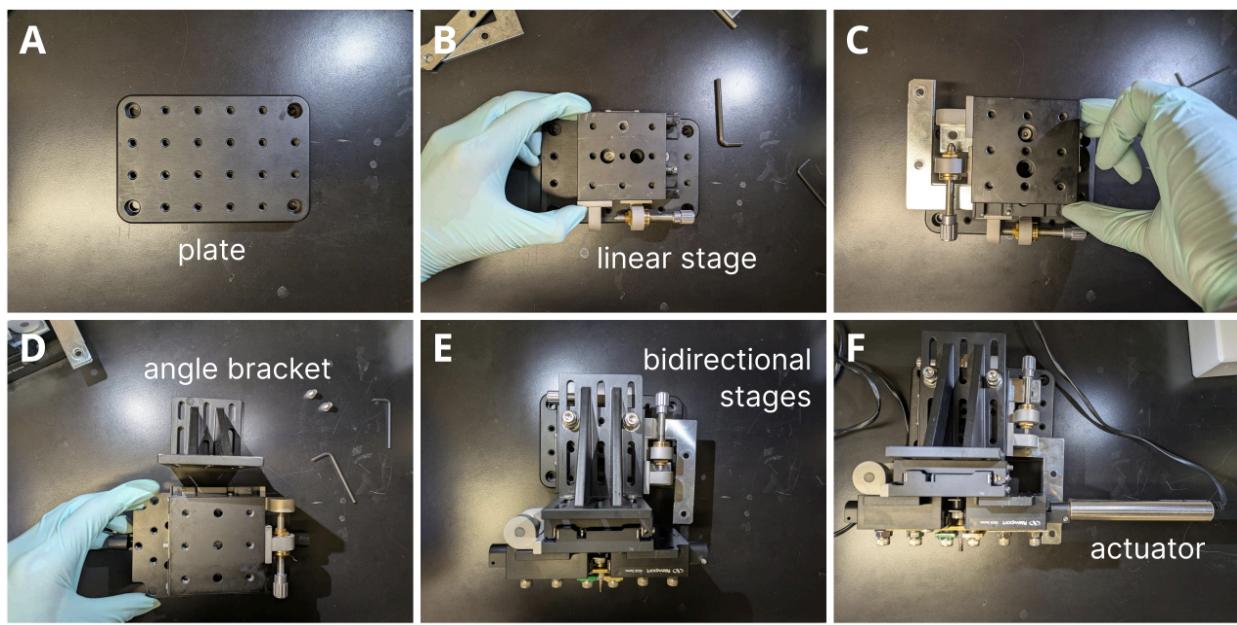
The device is to be used with an upright dissection or macroscope, enabling resolution of cell and tissue-scale tissue deformations under applied stretch, and a computer enabling control over the linear actuator that drives displacement of cantilevers to stretch the tissue and quantify associated forces. Additional details can be found in the manuscript by Oikonomou, Calvary and colleagues (2025).

### Assembly of the testing device

Broadly, the device is constructed through assembly of stages driven by linear actuators, and attachment of tungsten cantilevers to these stages so that they can be positioned in 3D, and then precisely moved along a single axis to perform stretching experiments. Here, we described the assembly of the device and attachment of the cantilevers.

#### *Assembly of linear actuator-driven stages for precise, bidirectional displacement*

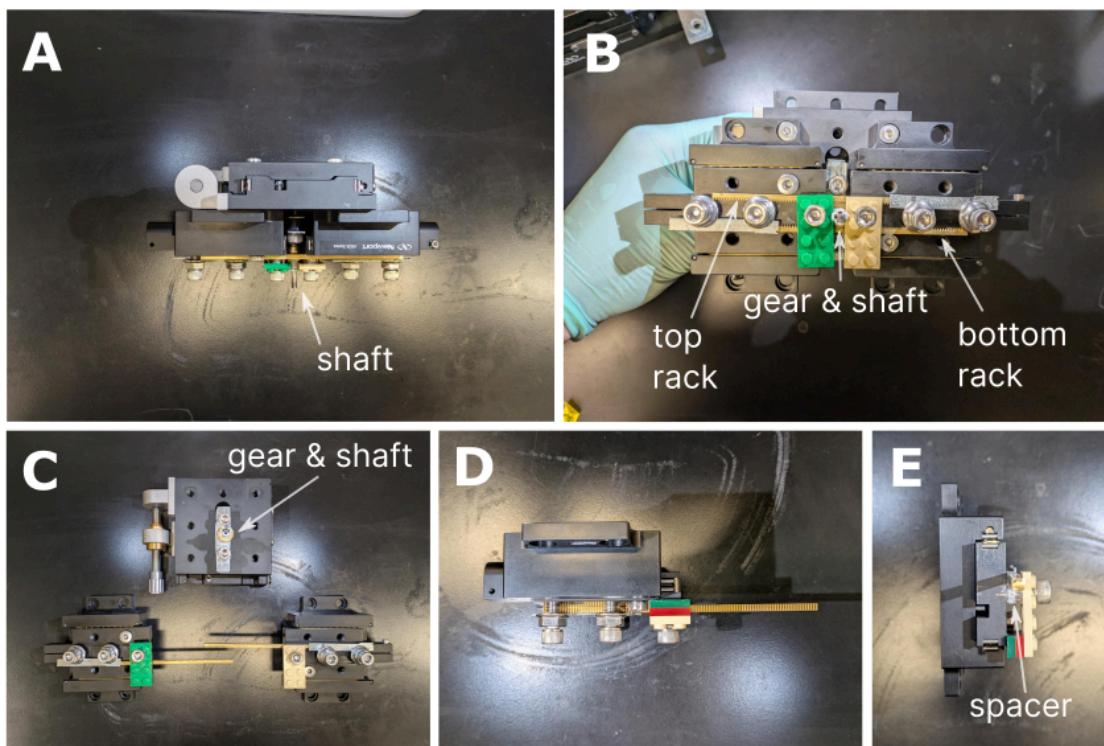
The core of the tensile tester is made up of four micrometer-driven linear stages (Newport Corporation #423-MIC). The first two are adjoined on a base plate to provide 2D planar control over position of the cantilevers (Fig. 1A-C). The third linear stage is mounted on these using an angle bracket (Newport #360-90) that orients it perpendicular to the base plate, enabling manual, coarse 3D positional adjustments (Fig 1D-E). A fourth linear stage was mounted on this setup with the purpose of providing precise, automated positional control along a single axis. The manual actuator on this stage was replaced with a DC servo linear actuator (Newport #CONEX-TRA25CC), which includes a controller that can interface with a computer for precision positional control via simple USB connection.



**Fig 1.** Step-by-step pictures of the assembly of the bidirectional mechanical tester

To convert unidirectional movement of the linear actuator into opposing movement of the two cantilever arms for bidirectional stretching, two Newport #460A-X linear stages were mounted back-to-back on the terminal micrometer stage so that each could translate in opposite directions. A single linear actuator drove one “active” stage outward; to drive the opposing “passive” stage without a second actuator, we interposed a gear train: a central brass gear (McMaster-Carr #7880K14), free to spin on a perpendicular steel shaft (McMaster-Carr #1327K93), meshed with two parallel brass rack gears (McMaster-Carr #7854K11), one fixed to each stage (Fig. 2B). As the actuator pushed the active stage (and its rack) rightward, the central gear reversed that motion into leftward travel of the passive rack. The shaft and gear assembly were housed in a chassis machined from a metal plate; spacer shims under the racks ensured smooth translation clearance, and edge-sanded bolts alongside the racks served as linear guides to prevent bowing under load.

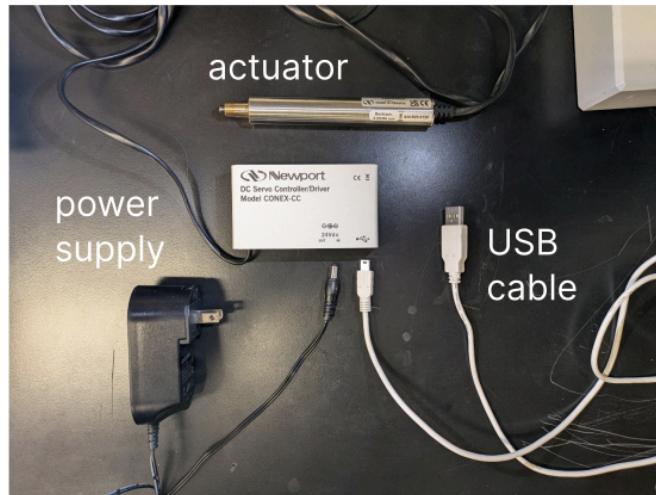
The fully assembled device was fastened to a solid aluminum optical breadboard (Newport #SA2-11), providing a stable base upon which experiments can be conducted.



**Fig 2.** Pictures of the bidirectional tester from two different perspectives, with key parts of the design labeled. In this case, the actuator was connected to the right stage, so it would drive the right stage/cantilever rightward, the right rack's motion would rotate the gear on the central shaft, and the gear would then transfer the motion to the left rack, thereby driving the left stage/cantilever leftward at the same speed. (D) and (E) are side views of the right stage (top rack).

#### Attachment of tungsten cantilevers to bi-directional linear actuator

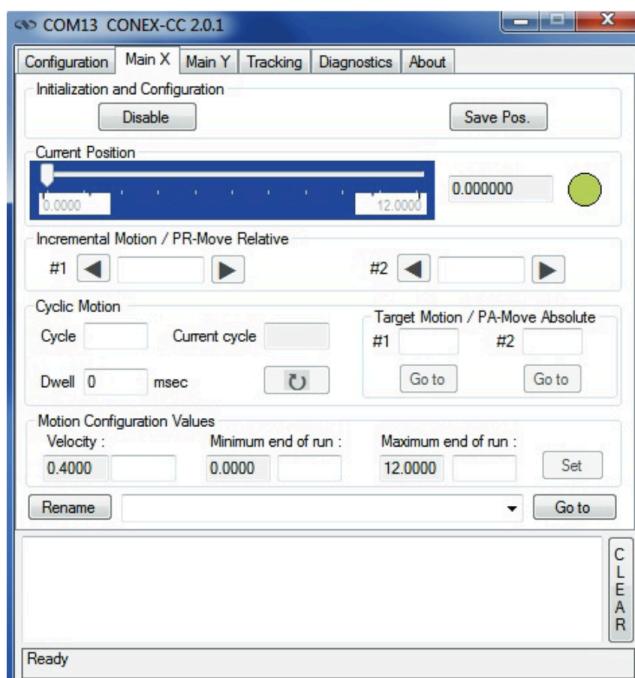
Movement of the linear actuator was translated to stretching of embryonic epithelia through attached cantilevers that engage filter paper grips on the embryo. As described in the main text, the deflection of these cantilevers is then used to compute the applied force. The cantilevers, made from fine tungsten wires, were mounted on the servo-controlled stage using a quick-swap LEGO interface to coarsely ensure cantilevers were in-line with the imaging plane. Each wire was extended by ~2 cm and pinched between a 2×2 stud LEGO brick and plate, clamping the wire securely. To integrate with the manipulator stages, holes were tapped in 2×4 LEGO stud plates to accept threaded bolts that fasten each plate—and thus the LEGO assembly—to the face of its respective linear stage (Fig 2B). Before installation, each bolt was slipped through the wide end of a 1000 µL pipette tip, which acted as a spacer to firmly secure it to the stage. Cantilevers are attached via lego pieces in an orientation parallel to the imaging plane, as shown in the main text Fig 1. A detailed description of cantilever length and diameter selection, as well as calibration, is available in the Methods section of the manuscript.



**Fig 3.** Newport CONEX controller and components, arranged to show how they connect to each other; Power supply (black, left), Actuator (silver, top) and USB A connection for computer control (white, right).

#### *Computer control of tensile tester*

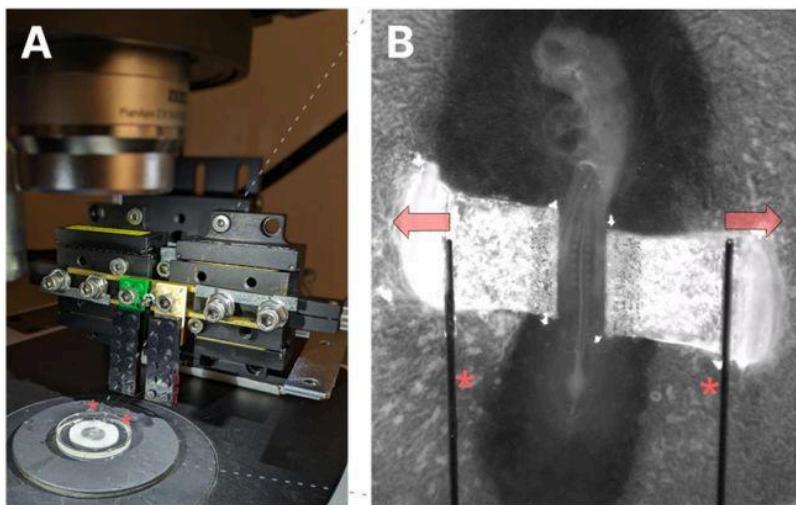
Above we have described the assembly of the device, and attachment of paired cantilevers whose precise position is under control of a DC servo linear actuator. Here, we explain the simple setup for running tensile testing experiments, which relies on bidirectional movement of the cantilevers, achieved through unidirectional movement of the actuator. The CONEX actuator is connected to a controller, which also receives a power supply and a micro-USB connector that is connected to a computer via type A USB (Fig. 3). A software provided by Newport, Newport CONEXCC Utility, can be downloaded and installed from the manufacturer, and has a simple user interface that provides a real time readout of actuator position, and the ability to input desired displacement rates, including both ramp and oscillatory movements (Fig. 4). For stretching experiments, a fixed velocity was input, and the test initiated by selection of the viscous forces (Fig 4). A detailed description of the software and its usage is available from the manufacturer ([https://www.newport.com/medias/sys\\_master/images/images/hc3/h1d/9044105035806/CONEX-CC-Controller-GUI-Manual.pdf](https://www.newport.com/medias/sys_master/images/images/hc3/h1d/9044105035806/CONEX-CC-Controller-GUI-Manual.pdf)).



**Fig 4.** The CONEX CC Controller GUI - of particular note is the 'Velocity' parameter.

## Mounting of embryos for stretching

Chick embryos are harvested onto filter paper rings for handling, as described in the main text, and remain on these filter papers throughout subsequent electroporation, incubation, and tensile testing on the apparatus (Fig. 5A). The mounting closely follows conventional EC culture, with embryos placed in 35 mm plastic culture dishes (08-772-30, Fisher Scientific) containing semisolid EC culture medium, a mixture of agar noble and albumin (see Methods). Unlike normal EC culture, however, the dishes are filled to the top with culture medium, facilitating the lateral access of cantilevers to engage the embryo. Finally, filter paper L-posts (Fig. 5B) can be placed directly on the tissue. These posts will be engaged by cantilevers to apply tension to the tissue, and therefore their size and placement should be carefully catered to the application. At this point, the culture dish can be placed directly on the base of the device (Fig. 5A). Once the sample is in place, stage control via manual linear actuators on the device can be used to position the cantilevers such that their bidirectional movement via the servo-driven actuator results in engagement of the L-posts and stretching of the tissue (Fig. 5B).



**Fig 5.** Sample mounting - reproduced from the original publication. (A) Sample atop the microscope stage - red asterisks denote the cantilevers. (B) Magnified view of cantilevers bilaterally engaging "L-shaped" filter papers adherent to the sample (ventral endodermal epithelium of the embryo).

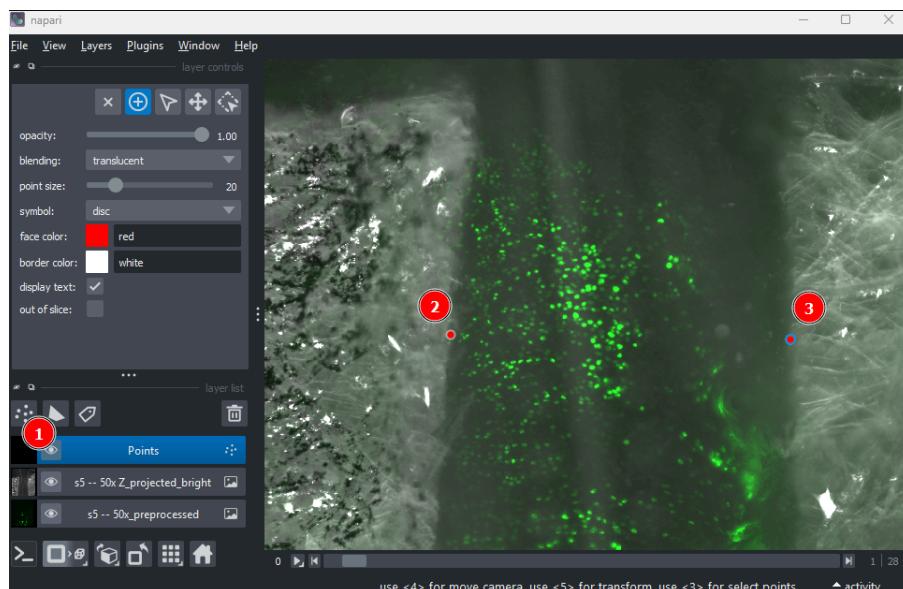
## Microscopy

All experiments have been conducted on a Zeiss Axiozoom macroscope, though the device is compatible with any upright microscope with a working distance of 0.5 mm or more. Additional hardware can be used to extend the cantilever mount to accommodate a range of other microscope setups and objective sizes if needed. Owing to the weight of the base and the small scale of forces applied, the device sits stably atop the microscope stage without need for any additional installation or coupling. The microscope was operated using Zen, the Zeiss commercial software, installed on the same computer running the CONEX software to control the actuator. Using the time lapse function in Zen, image collection was initiated immediately upon the start of actuator displacement to synchronize image collection actuator displacement; this is particularly important for quantification of cantilever deflection.

## Data acquisition and analysis

The primary data format of this technique is imaging, as all measures, including force, stretch, and all cell morphometric parameters were extracted from time lapse images of stretching experiments, when combined with the user-input velocity of the cantilever base. To correct for subtle differences in the focal plane, the z-stack function was used in Zen at each time point in the time lapse to collect multiple focal planes, which were subsequently combined through focus-correction using the `extended\_depth\_of\_focus.py` script.

From the corrected images, the next step is to extract the position of the filter paper edge in each frame of the time lapse. This was done manually in napari using the points function (Fig 6), but could be done in Fiji as well. Point annotations were then exported in a .csv file, where each row is a point and each column has a coordinate (time, y, x).



**Fig 6.** Point annotation (fiducial markers) using the napari GUI - alternatively Fiji or any other image analysis software can be used, so long as you can export the annotations as a .csv file.

Next, we describe how custom code included online ([Calculate strain and stress.py](#)) is used to process these data. To read paired coordinate CSVs, `get_distance` parses a file into a Pandas DataFrame, groups rows by time step, and extracts two points per frame. We then compute the separation distance using `eucledian_distance_2d`. To convert raw distance measurements into normalized deformation metrics, the function `get_strain` computes Lagrangian strain. The utility `eucledian_distance_2d` returns the Euclidean distance between two 2D points  $(x_1, y_1)$  and  $(x_2, y_2)$ . Translating strain into physical deflection and force, `get_deflection_from_strain` uses the input actuator speed, time lapse frame capture rate, and initial length to compute beam deflection over time. `get_cantilever_force_from_deflection` then converts deflection values into force via a spring constant `stiffness_k`. Note that users will have to input the strain rate of the device and the seconds between each successive frame in the movie, and the cantilever stiffness such that forces are calculated.

Several Xarray-focused utilities streamline working with labeled multidimensional data. The function `find_low_unique_var` identifies a coordinate variable with low cardinality (e.g., a Z-axis). `annotate_names_to_xarray` renames ambiguous `axis-*` or generic columns to meaningful labels like `X_POS`, `Y_POS`, and `TIME`. The paired functions `calculate_distance`

and `compute_strain_and_rolling_avg` then compute per-frame distances, engineer both simple and Lagrangian strain definitions, and apply a two-point rolling average.

Analysis of the results of cell segmentation and tracking are performed in the notebook (`Cell_tracks_and_morphometrics_analysis.ipynb`). This notebook implements a complete pipeline for analyzing cell tracking outputs and morphometric features, beginning with data loading and ending with clustering, visualization, and statistical comparisons. Once dependencies are in place, the notebook mounts Google Drive and prompts the user to specify a `Data_folder` path. It lists all files in this directory and applies a regular-expression filter (`regex_pattern`) to select CSV files containing the cell tracks (btrack).

Feature engineering begins by encoding textual `sample_id` values into numeric codes via `LabelEncoder` and constructing a unique track identifier `ID_unique`. The notebook computes a `shape_index` as the ratio of `perimeter` to the square root of `area`. It then groups tracks by `ID_unique` and applies the functions `compute_area_zero` and `compute_last_area` to assign each track the mean area of its first three and last three timepoints, respectively. From these, new columns `area_norm` and `last_to_initial_area` are derived to normalize growth measures. The tracks can be further filtered for quality (e.g. discard tracks with unreasonable area variation due to segmentation or tracking errors). In the UMAP analysis section, the user chooses a list of morphometric features to create a 2D embedding for exploratory analysis. Clustering is performed with HDBSCAN by instantiating `hdbscan.HDBSCAN` with user-defined `min_samples`, `min_cluster_size`, and `metric`. Feature distributions within clusters and across conditions are visualized using `sns.boxplot` and `sns.stripplot`, highlighting differences in initial area, aspect ratio, and other metrics.

Temporal dynamics of morphometric changes are examined by defining an interpolating function and applying it per track to sample feature trajectories across a defined strain range. Smoothed mean and standard deviation curves are then plotted for each cluster or condition, optionally filtered and smoothed further using `savgol_filter` from SciPy.

Statistical comparisons include one-way ANOVA via `stats.f_oneway`, followed by post-hoc Tukey HSD tests using `pairwise_tukeyhsd`. For adjusted models accounting for covariates, the notebook fits an ANCOVA with `ols` from `statsmodels.formula.api` and computes type-II sums of squares using `anova_lm`.