Supplementary Information - MechanoBioCAD: A Generalized Semi-Automated Computational Tool for Mechanobiological Studies

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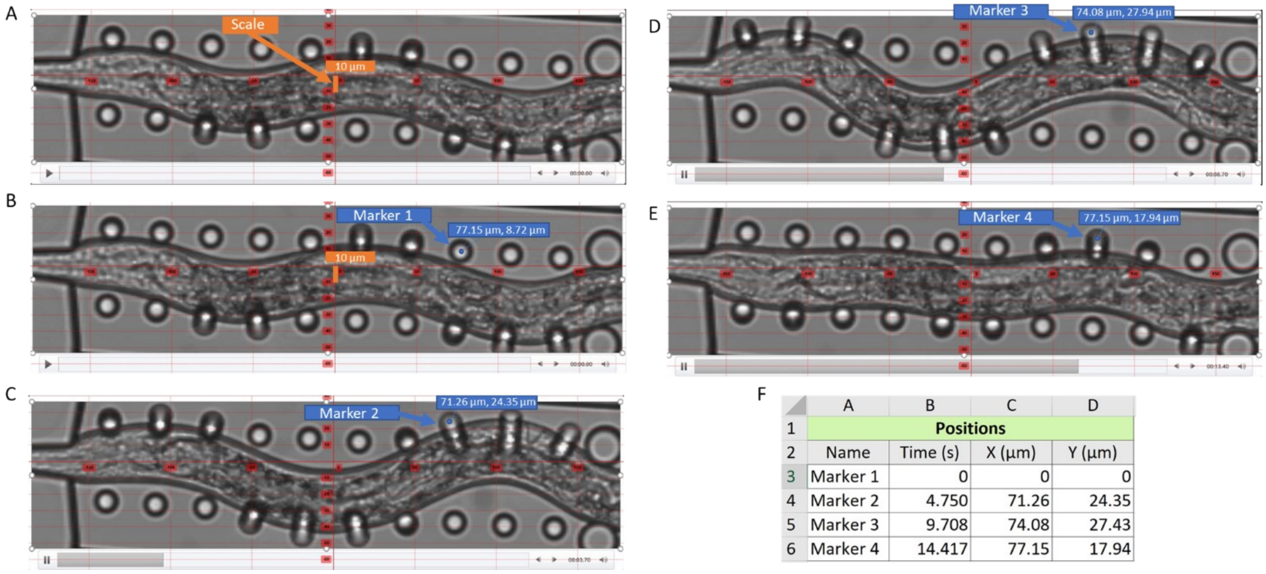
Supplementary Text 1

Estimation of X- and Y-Displacement for MBC Analysis



	A	B	C	D
1	**Positions**			
2	Name	Time (s)	X (µm)	Y (µm)
3	Marker 1	0	0	0
4	Marker 2	4.750	71.26	24.35
5	Marker 3	9.708	74.08	27.43
6	Marker 4	14.417	77.15	17.94

Fig. S1: Step-by-step measurement of micropillar displacements using Kinovea software. A, Calibration of the scale to 10 µm and setting the origin at the top of the scale line. B, Marker 1 placed at the center of the micropillar in its undeformed state. C-E, Markers 2, 3, and 4 placed at the center of the micropillar in its deformed states at different frames, capturing X and Y coordinates. F, Exported displacement data (X, Y coordinates) in an Excel file for further analysis.

To extract the X and Y displacements of the deformed micropillar, begin by downloading and installing Kinovea[1], an open-source software for motion tracking. Load the video of the micropillar deformation into the software to proceed with the analysis. The process starts by drawing a vertical line in the image and calibrating it to represent a scale of 10 µm, as shown in Fig. S1A. This ensures precise alignment with the image axes for accurate deformation measurements. The origin is established at the top of the scale line, serving as a consistent reference point for subsequent analyses. In Fig. S1B, Marker 1 is placed at the center of the micropillar in its undeformed state, and its X and Y coordinates are recorded as the baseline for comparison. During deformation, as depicted in Figs. S1C-E, markers (labeled as Markers 2, 3, and 4) are placed at the center of the micropillar in its deformed states across different video frames, capturing the X and Y coordinates corresponding to various deformation stages. Finally, as illustrated in Fig. S1F, all markers' displacement data (X, Y coordinates) are exported into an Excel file. This data can be formatted for direct input into the MBC software for further analysis. Additionally, Kinovea and similar motion-tracking software provide features like continuous point tracking across video frames, enabling the generation of a comprehensive dataset of X and Y coordinates over time or frame-by-frame. This facilitates a detailed and efficient analysis of micropillar deformation.

Force Estimation Using MBC

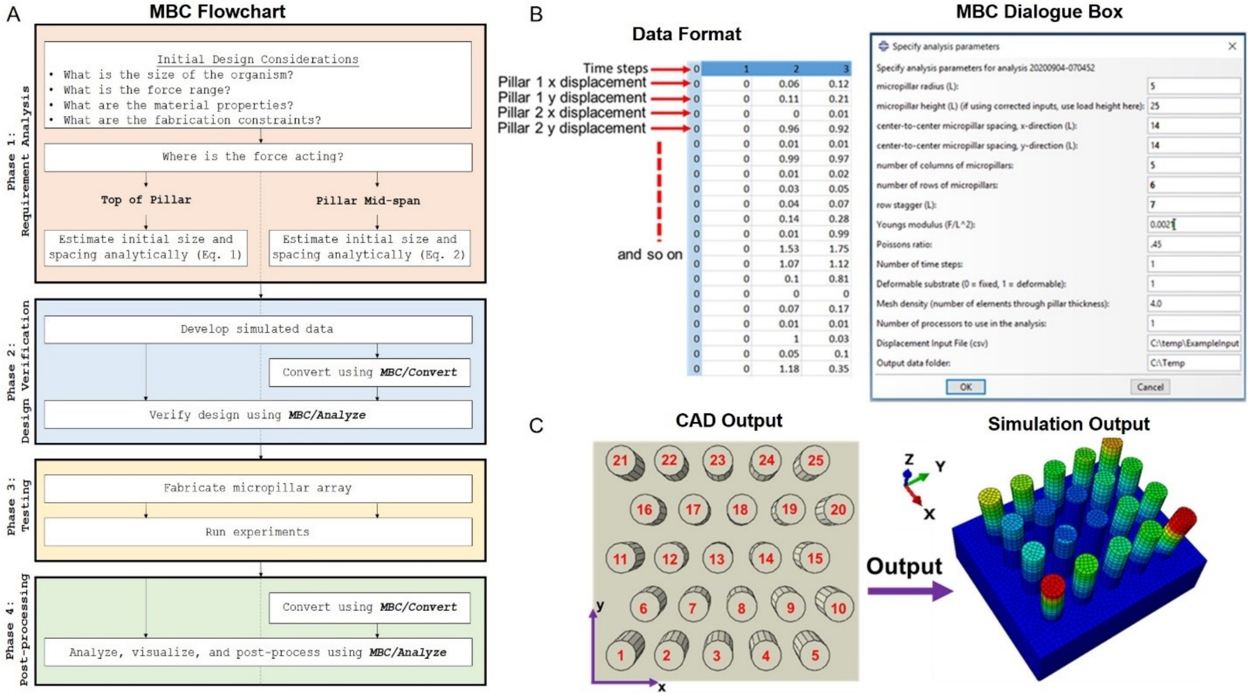
Fig. S2A shows an overview of the MBC workflow. The MBC protocol consists of four phases: Requirement Analysis, where study parameters are defined, Design Verification, where simulated data is analyzed for substrate warping and non-linearity, Testing, where micropillar arrays are fabricated and experimental data is collected, and Post-Processing, where the data is analyzed using stress contour plots to assess forces and interactions. The details of each phase are provided below.

Phase 1 - Requirement Analysis: In the preliminary stage of the MBC protocol, the workflow begins with initial design considerations, focusing on outlining the study's primary characteristics. This involves specifying the organism size, defining the force range that will be tested, identifying the material properties of micropillars, and acknowledging any limitations posed by fabrication techniques or financial resources. This stage is pivotal as it ensures that the micropillars are designed to meet the exact requirements of the study.[2,3]

Phase 2 - Design Verification: This phase comprises design verification through the analysis of simulated data using MBC/Analyze. First, the user inputs the corresponding displacements and all the required inputs based on the specifications, as shown in Fig. S2B. Upon completion, MBC provides the corresponding force values and associated stress contour plots. Using these contour plots, the user can check for substrate warping/deformation, interpillar effects affecting neighboring pillars, and potential non-linear effects caused by pillar deflection. Most cases employ top-view microscopy-based displacement estimation, which tracks the pillar's top face due to the relatively simple image analysis involved. This method makes it straightforward to obtain displacement values for traction at the top of the pillar, as seen with cells.

However, an additional step is required when assessing mid-span displacement, as is the case with multicellular organisms such as C. *elegans*. This necessity arises because mid-span pillar displacement differs from that of the top. To obtain the correct displacements at the mid-span, MBC/Convert can be used, as it adjusts the given displacement values for mid-span loading using a regression model, making them compatible with the analysis in MBC/Analyze. The FEM codes of MBC/Analyze and MBC/Convert can be downloaded as open-source codes from the online site[4]. Due to changes in the Python library structure between different Abaqus editions, we have provided two versions of each code—one for Abaqus 2022 and earlier and another for Abaqus 2023 and later.

Phase 3 - Testing: After completing the design verification process, physical micropillar arrays are fabricated for experimental testing. Experimental tests are done using cells or any animal model where the micropillar displacements are measured using 2D microscopy. The experimental displacement data are subsequently utilized for post-processing and error estimation, as outlined in Phase 4.



0	1	2	3
0	0	0.06	0.12
0	0	0.11	0.21
0	0	0	0.01
0	0	0.96	0.92
0	0	0.01	0.01
0	0	0.99	0.97
0	0	0.01	0.02
0	0	0.03	0.05
0	0	0.04	0.07
0	0	0.14	0.28
0	0	0.01	0.99
0	0	1.53	1.75
0	0	1.07	1.12
0	0	0.1	0.81
0	0	0	0
0	0	0.07	0.17
0	0	0.01	0.01
0	0	1	0.03
0	0	0.05	0.1
0	0	1.18	0.35

Specify analysis parameters

Specify analysis parameters for analysis 20200904-070452

micropillar radius (L):	5
micropillar height (L) (if using corrected inputs, use load height here):	25
center-to-center micropillar spacing, x-direction (L):	14
center-to-center micropillar spacing, y-direction (L):	14
number of columns of micropillars:	5
number of rows of micropillars:	6
row stagger (L):	7
Youngs modulus (F/L^2):	0.002
Poissons ratio:	.45
Number of time steps:	1
Deformable substrate (0 = fixed, 1 = deformable):	1
Mesh density (number of elements through pillar thickness):	4.0
Number of processors to use in the analysis:	1
Displacement Input File (csv)	C:\temp\ExampleInput
Output data folder:	C:\Temp

Fig. S2: Overview of MBC workflow. A, MBC workflow flowchart in four phases. B, MBC analysis procedure. The comma separated values (CSV) file should follow the given format where the first column contains only zero while the first row contains the time steps of simulation. Dialogue box showing the analysis parameters that needed to be entered for geometry, pillar arrangement, row stagger distance, material properties, time steps, deformable vs fixed substrate, mesh density as well for the CSV file input and result file's output location. C, Generated pillar array in the part module of MBC and the associated meshed visualization output based on the input parameters. The axes and the pillar numbering scheme are shown.

Phase 4 - Post-Processing: In the final phase, the experimental displacement data are directly imported into MBC/Analyze for cells. Additionally, MBC/Convert can be used to convert the experimental displacement data for animal models, such as C. *elegans*, particularly for the mid-span case, into a format compatible with MBC/Analyze. Subsequently, MBC/Analyze is utilized to visualize and comprehensively analyze the force responses using associated stress contour plots. This includes comparisons with other cases, such as deformable versus fixed substrate for the substrate effect, top versus mid-span height for the effect of incorrect height assumption, the effect of non-linearity, and interpillar effects (i.e., the effect from neighboring pillars).

The graphics user interface (GUI) shown in Fig. S2B solicits critical information such as the geometric parameters of the micropillars (radius and height), their spatial arrangement (inter-pillar spacing in the x and y directions and row stagger for eccentric hexagonal layouts), and material properties (Young's modulus and Poisson's ratio). The user is also presented with the choice to designate the substrate as either rigid (0) or deformable (1), a selection that influences the resulting force calculations. Additionally, the figure shows the data format of the comma-separated values (CSV) file that needs to be uploaded to MBC. Mesh density is another critical input, with the entered value determining the granularity of the finite element mesh across both the micropillar and the substrate. For example, Fig. S2C displays 25 micropillars configured according to the given parameters: 2.5 μm diameter, 7.5 μm height, and 4 μm center-to-center spacing in both x- and y-directions, with a 2 μm staggered offset between rows. This setup details the number of elements per pillar and the substrate thickness. The figure also outlines the directional axes and the numbering system used for the micropillars. The Abaqus visualization module highlights the displacement contour plot, where the intensity of red indicates the maximum deflection. The resulting output, a text file in a specific location, contains the calculated reaction forces for each micropillar, which can be accessed from the chosen input directory.

Supplementary Text 2

Modulus estimation

We estimated Young's modulus of the PDMS that we cured at 60 °C for 12 hours using a cantilever based free end loading experiment as seen in Fig. S3A. The geometry used was 50 mm in height and 5 mm in diameter. The free-end was deflected using the displacement rate of 1 mm/s and the output was recorded in the form of force vs deflection curve, which was linearly fitted to calculate the slope, as seen in Fig. S3B. The slope was equated to $\frac{3EI}{L^3}$ (slope from the Euler-Bernoulli beam equation for free end-loaded cantilever beam). Young's modulus (E = 2.1 MPa) was calculated by inserting the associated area moment of inertia (I) and length (L) values.

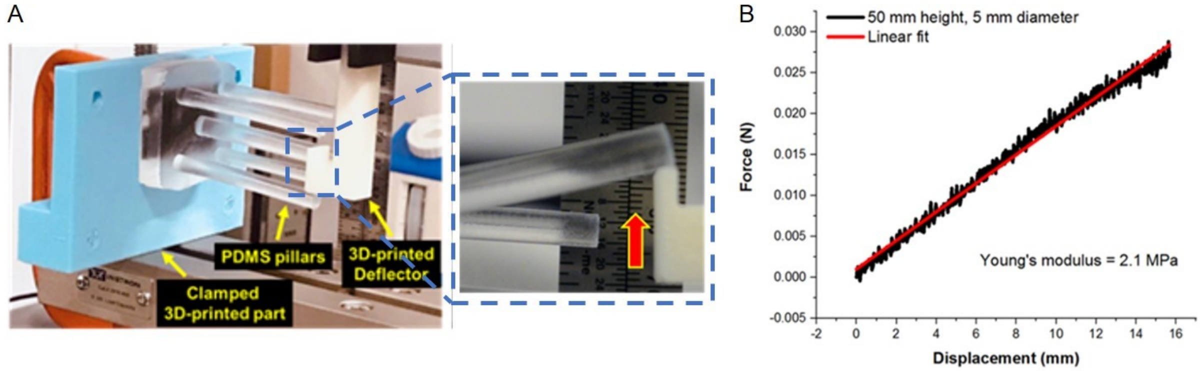


Fig. S3. Young's modulus of elasticity estimation. A, Experimental setup to estimate Young's modulus of the cured PDMS using cantilever beam free end loading. The pillar height and diameter were 50 mm and 5 mm, respectively. B, The results obtained from the modulus estimation test. The Young's modulus estimated was 2.1 MPa.

Supplementary Text 3

MBC as an Open-Source Tool with ABAQUS Student Edition

After downloading and installing the ABAQUS Student Edition from the website, open the software. On the start session screen, click on "With Standard/Explicit Model" to create a new model database, as depicted in Fig. S4. This will open the main ABAQUS/CAE interface. Next, download the MBC Python script from the online site[4] and save it to a known location on your computer. In the ABAQUS/CAE interface, go to the "File" menu and select "Run Script," as shown in Fig. S5. A file browser window will appear, allowing you to navigate to the location where you saved the MBC Python script.

Select the script and click "Open" to load and execute it in ABAQUS. After loading the MBC code, users are prompted to input critical information using the graphics user interface (GUI) shown in Fig. S6. This includes the geometric parameters of the micropillars (radius and height), their spatial arrangement (inter-pillar spacing in the x and y directions and row stagger for eccentric hexagonal layouts), and material properties (Young's modulus and Poisson's ratio). Users are also given the option to designate the substrate as either rigid (0) or deformable (1), which influences the resulting force calculations. Additionally, the figure shows the data format of the comma-separated values (CSV) file that needs to be uploaded to MBC. Mesh density is another essential input, with the entered value determining the granularity of the finite element mesh across both the micropillar and the substrate. An example input file (Input Example.csv) is also available for quick testing and will run successfully in the Student Edition if the mesh size is set to 0.5 or less, keeping within the 1000-node limit of the Student Edition.

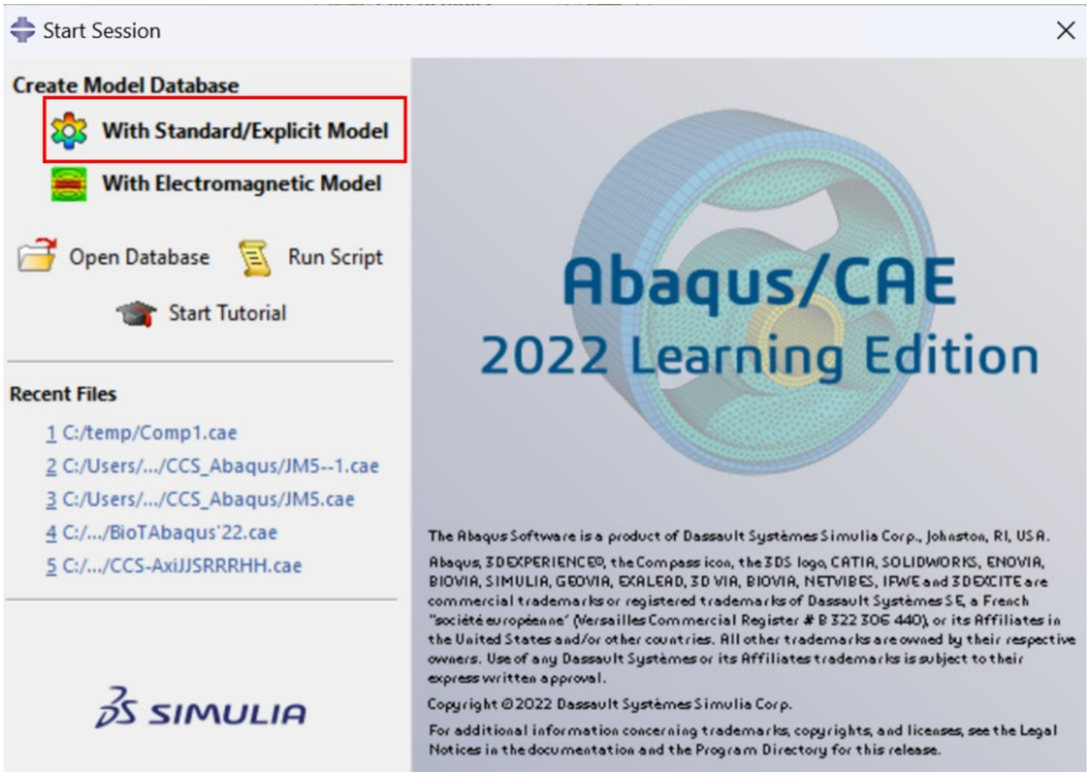


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Fig. S4. ABAQUS Standard/Explicit Model. MBC is specifically designed for micropillar-based biomechanics applications and thus works with the ABAQUS Standard/Explicit Model.

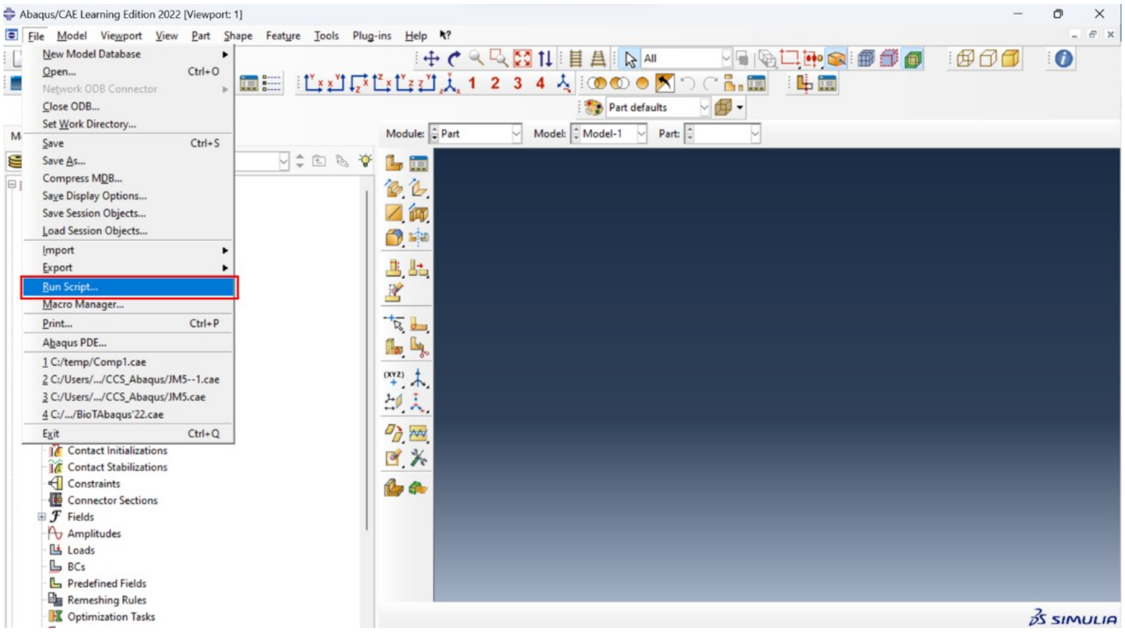
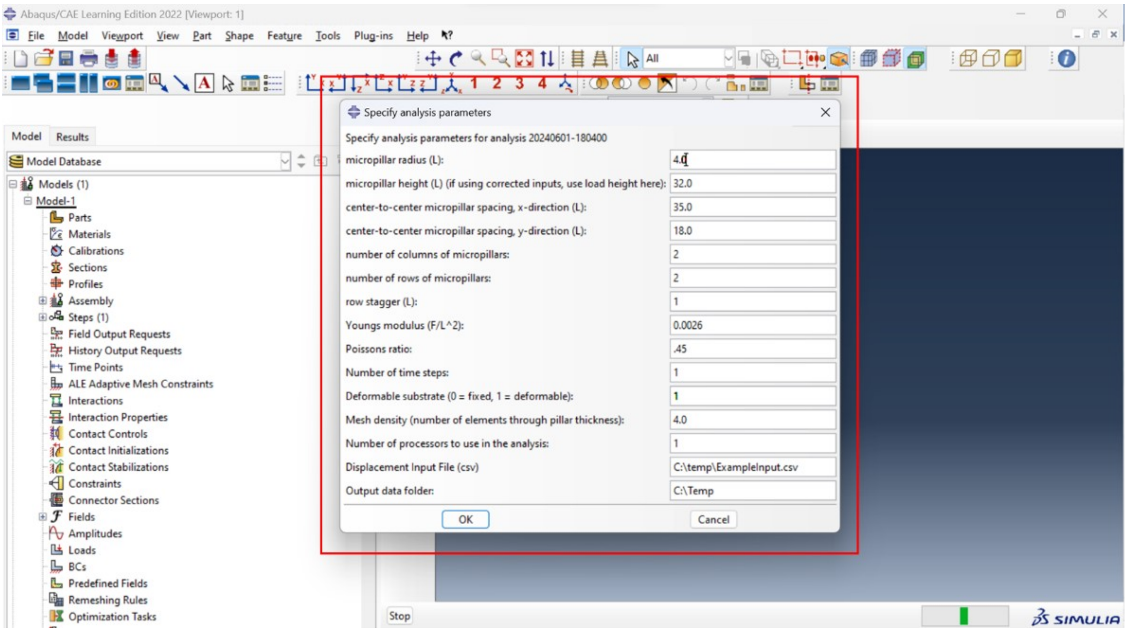


Fig. S5. Running the MBC Script in ABAQUS. After opening the ABAQUS/CAE interface, users can load the MechanoBioCAD (MBC) script by navigating to the "File" menu and selecting "Run Script."



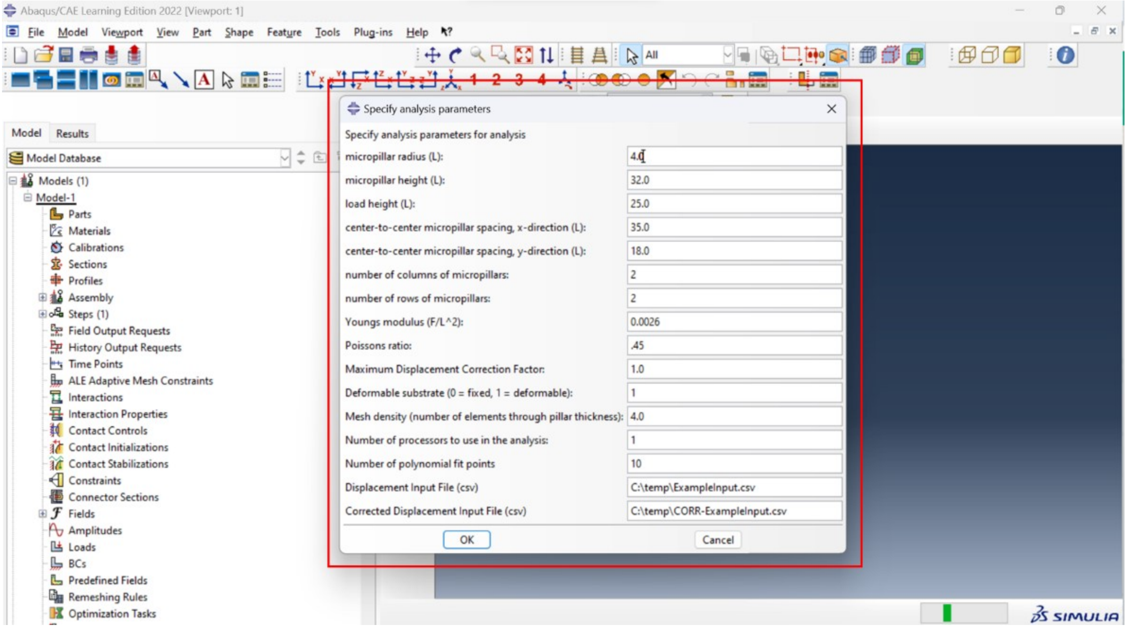
Specify analysis parameters

Specify analysis parameters for analysis 20240601-180400

micropillar radius (L):	4.0
micropillar height (L) (if using corrected inputs, use load height here):	32.0
center-to-center micropillar spacing, x-direction (L):	35.0
center-to-center micropillar spacing, y-direction (L):	18.0
number of columns of micropillars:	2
number of rows of micropillars:	2
row stagger (L):	1
Youngs modulus (F/L^2):	0.0026
Poissons ratio:	.45
Number of time steps:	1
Deformable substrate (0 = fixed, 1 = deformable):	1
Mesh density (number of elements through pillar thickness):	4.0
Number of processors to use in the analysis:	1
Displacement Input File (csv)	C:\temp\ExampleInput.csv
Output data folder:	C:\Temp

Fig. S6. Specifying Analysis Parameters in ABAQUS. After running the MBC script, users are prompted to input critical analysis parameters.

Similarly, for MBC/Convert, users need to enter all relevant details regarding the animal model, such as C. elegans. The interface, as shown in Fig. S7, prompts users to input the necessary parameters, including micropillar geometric properties, spatial arrangement, material properties, and mesh density. Users then upload the CSV file containing the top-view displacements. The software processes this data and provides an output CSV file with corrected displacements at the mid-span. This corrected data is crucial for accurately analyzing the mechanical behavior of multicellular organisms where mid-span displacements differ from top-view observations. The corrected CSV file should then be used in MBC/Analyze to proceed with the rest of the analysis as intended.



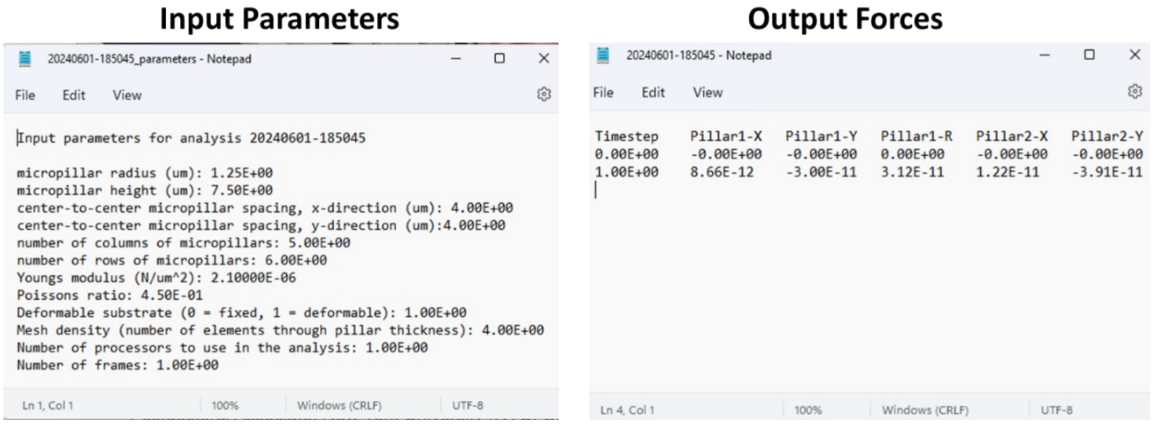
Specify analysis parameters

Specify analysis parameters for analysis

```
micropillar radius (L): 4.0
micropillar height (L): 32.0
load height (L): 25.0
center-to-center micropillar spacing, x-direction (L): 35.0
center-to-center micropillar spacing, y-direction (L): 18.0
number of columns of micropillars: 2
number of rows of micropillars: 2
Youngs modulus (F/L^2): 0.0026
Poissons ratio: .45
Maximum Displacement Correction Factor: 1.0
Deformable substrate (0 = fixed, 1 = deformable): 1
Mesh density (number of elements through pillar thickness): 4.0
Number of processors to use in the analysis: 1
Number of polynomial fit points 10
Displacement Input File (csv) C:\temp\ExampleInput.csv
Corrected Displacement Input File (csv) C:\temp\CORR-ExampleInput.csv
```

Fig. S7. Specifying Parameters in MBC/Convert. The interface prompts users to enter parameters and upload the CSV file containing top-view displacements, generating an output CSV file with corrected mid-span displacements.

After executing the code, MBC/Analyze generates two text document files (Fig. S8). The first file contains the input parameters, allowing the user to verify all the parameters entered. The second file provides detailed results, listing the pillar numbers along with their associated x, y, and resultant components of the forces.



Input Parameters

```
Input parameters for analysis 20240601-185045

micropillar radius (um): 1.25E+00
micropillar height (um): 7.50E+00
center-to-center micropillar spacing, x-direction (um): 4.00E+00
center-to-center micropillar spacing, y-direction (um):4.00E+00
number of columns of micropillars: 5.00E+00
number of rows of micropillars: 6.00E+00
Youngs modulus (N/um^2): 2.10000E-06
Poissons ratio: 4.50E-01
Deformable substrate (0 = fixed, 1 = deformable): 1.00E+00
Mesh density (number of elements through pillar thickness): 4.00E+00
Number of processors to use in the analysis: 1.00E+00
Number of frames: 1.00E+00
```

Output Forces

```
Timestep   Pillar1-X  Pillar1-Y  Pillar1-R  Pillar2-X  Pillar2-Y
0.00E+00   -0.00E+00  -0.00E+00  0.00E+00   -0.00E+00  -0.00E+00
1.00E+00   8.66E-12   -3.00E-11  3.12E-11   1.22E-11   -3.91E-11
```

Fig. S8. Output Files Generated by MBC/Analyze. The first file shows the input parameters, while the second file lists the pillar numbers and their corresponding x, y, and resultant force components.

References

1 Kinovea, https://www.kinovea.org/, (accessed 20 January 2025).

2 S. Sofela, Y. Feng, N. S. Baban, C. J. Stubbs, Y.-A. Song and W. Wang, in *Micro and Nano Systems for Biophysical Studies of Cells and Small Organisms*, Academic Press, 2021, pp. 261–293.

3 I. Schoen, W. Hu, E. Klotzsch and V. Vogel, *Nano Lett.*, 2010, **10**, 1823–1830.

4 N. S. Baban, MechanoBioCAD, https://sites.google.com/nyu.edu/singhnavajit/research?authuser=0, (accessed 2 June 2024).