

TractionsForAll

v1.0

September 1st, 2013

TractionsForAll v1.0 is freely distributed program that calculates tractions exerted by an adherent cell on the soft hydrogel substrate underneath. It is created to allow the students and researchers in the field of mechanobiology to study contractile responses of variety of cell types. Although helpful for understanding the process, no background in applied mechanics and computer programming is needed for using the software. The program is written and compiled in Matlab (Mathworks).

More details about the background theory and implemented experimental techniques can be found in following references¹⁻⁴:

1. Marinković A, Mih JD, Park JA, Liu F, Tschumperlin DJ. [Improved throughput traction microscopy reveals pivotal role for matrix stiffness in fibroblast contractility and TGF-beta responsiveness.](#) American journal of physiology Lung cellular and molecular physiology 2012; 303:L169-80.
2. Mih JD, Sharif AS, Liu F, Marinković A, Symer MM, Tschumperlin DJ. [A multiwell platform for studying stiffness-dependent cell biology.](#) PloS one 2011; 6:e19929.
3. Butler JP, Tolic-Norrelykke IM, Fabry B, Fredberg JJ. [Traction fields, moments, and strain energy that cells exert on their surroundings.](#) American journal of physiology 2002; 282:C595-605.
4. Tolic-Norrelykke IM, Butler JP, Chen J, Wang N. [Spatial and temporal traction response in human airway smooth muscle cells.](#) American journal of physiology 2002; 283:C1254-66.

If you find this program useful please cite these papers in your publication.

Traction calculation in few simple steps

Outlook of the main window

TractionsForAll

File

Current Folder: D:\Experiments\test01\ **Load First Image**

Crop Images & Camera Settings

Crop Images

ROI Size (px) Update

Pixel Size (um)

Objective Magnification

Displacement Calculation Settings

Initial Block Size (px)

Resolution Step (px)

Pixel Intensity Cut-off

XCorrelation Threshold

Max. Iteration Steps

Interpolation Method

Gel Properties

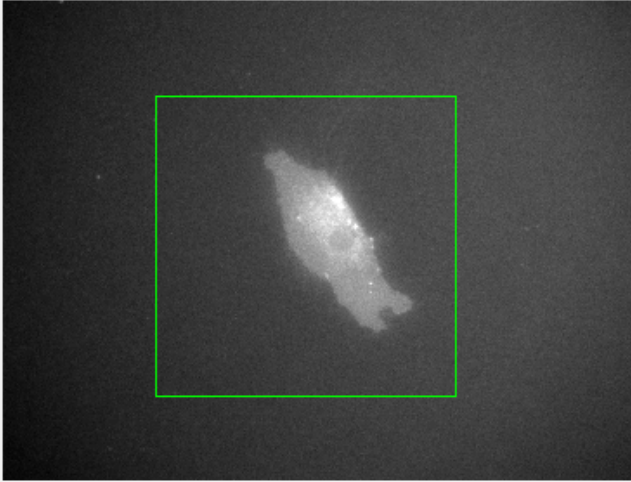
Elastic Modulus (Pa)

Poisson's Ratio

Analysis

Calculate Displacements

Calculate Tractions

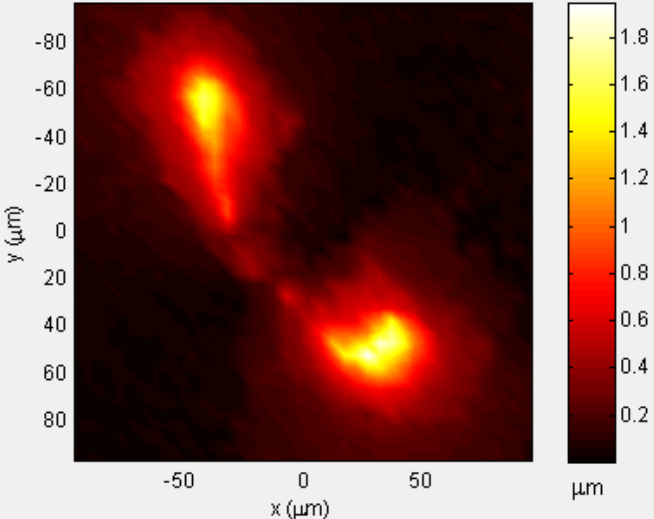


Constrained t...		Principal mo...	
Contraction ...		Orientation o...	x*x* principal... y*
Mxx	Myy	Mxy	
-27.31907	-47.93951	-29.36820	54.67226
			-68.75471
			-6
Centroid coo...		Second mom...	
x_bar (um)	y_bar (um)	bx_bar (um^4)	lyy_bar (um^4)
100.24784	100.91930	3641007.960...	1909485.904...
		2004761.484...	56

Cell Boundary

Outline Cell

Displacements



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Pick a Map Export

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1. Select a sequence of images for analysis ✓

File

Current Folder

Load First Image ✓

Crop Images & Camera Settings

ROI Size (px)

Pixel Size (um)

Objective Magnification

Displacement Calculation Settings

Initial Block Size (px)

Resolution Step (px)

Pixel Intensity Cut-off

XCorrelation Threshold

Max. Iteration Steps

Interpolation Method

Gel Properties

Elastic Modulus (Pa)

Poisson's Ratio

Analysis

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First cell image in the sequence should be named:

phase01.tif

All subsequent images should have names:

phase02.tif, phase03.tif, phase04.tif...

Corresponding images of fluorescent beads should be named:

image01.tif, image02.tif, image03.tif, image04.tif...

Reference image of fluorescent beads on unstressed gel

surface should have name: **trypsin.tif**

Note: All images have to be located in the same folder.

2. Crop the images to a square shaped format ✓

Current Folder: D:\Experiments\test\01\ Load First Image

Crop Images & Camera Settings

Crop Images

ROI Size (px) 640 Update

Pixel Size (um) 6.211

Objective Magnification 20

Displacement Calculation Settings

Initial Block Size (px) 32

Resolution Step (px) 16

Pixel Intensity Cut-off 125

XCorrelation Threshold 0.65

Max. Iteration Steps 20

Interpolation Method v4

Gel Properties

Elastic Modulus (Pa) 20000

Poisson's Ratio 0.48

Analysis

Calculate Displacements

Calculate Traction

Region Of Interest (ROI) size should be large enough to contain entire cell.

Note: Larger image size → Longer calculation

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3. Drag the square and double click to select the region of interest ✓

The screenshot displays the TractionsForAll software interface. At the top, the 'File' menu is visible. Below it, the 'Current Folder' is set to 'D:\Experiments\test\01\'. A 'Load First Image' button is present. The main workspace shows a grayscale image of a cell with a blue square region of interest (ROI) selected. A yellow checkmark is placed on the right side of the ROI, and a yellow mouse cursor is positioned at the bottom right corner of the square. Above the image, the coordinates '[312 201 640 640]' are displayed in pink. The left sidebar contains several settings panels: 'Crop Images & Camera Settings' with a 'Crop Images' button and fields for ROI Size (px) 640, Pixel Size (um) 6.2111, and Objective Magnification 20; 'Displacement Calculation Settings' with fields for Initial Block Size (px) 32, Resolution Step (px) 16, Pixel Intensity Cut-off 125, XCorrelation Threshold 0.65, Max. Iteration Steps 20, and Interpolation Method 'v4'; and 'Gel Properties' with fields for Elastic Modulus (Pa) 20000 and Poisson's Ratio 0.48. At the bottom left, there are two buttons: 'Calculate Displacements' and 'Calculate Traction'. A red text overlay 'SELECT A REGION!' is positioned above these buttons. The bottom of the window contains copyright information: '© 2013, Created by Aleksandar Marinkovic, Sc.D., Massachusetts General Hospital & Daniel Tschumperlin, Ph.D., Mayo Clinic'.

Current Folder: D:\Experiments\test\01\

Load First Image

[312 201 640 640]

Crop Images & Camera Settings

Crop Images

ROI Size (px) 640 Update

Pixel Size (um) 6.2111

Objective Magnification 20

Displacement Calculation Settings

Initial Block Size (px) 32

Resolution Step (px) 16

Pixel Intensity Cut-off 125

XCorrelation Threshold 0.65

Max. Iteration Steps 20

Interpolation Method 'v4'

Gel Properties

Elastic Modulus (Pa) 20000

Poisson's Ratio 0.48

SELECT A REGION!

Analysis

Calculate Displacements

Calculate Traction

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Note: Cropped image may be shifted a little bit since an algorithm that corrects the stage drift is applied.

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4. Outline a cell on the cropped image ✓

The screenshot displays the TractionForAll software interface. At the top, the 'File' menu is visible. Below it, the 'Current Folder' is set to 'D:\Experiments\test\01\'. A 'Load First Image' button is present. The main workspace shows a grayscale image of a cell with a green rectangular outline. Above the image, the coordinates '[312 201 640 640]' are displayed. To the left of the image are several settings panels: 'Crop Images & Camera Settings' (with a 'Crop Images' button, ROI Size of 640 px, Pixel Size of 6.2111 um, and Objective Magnification of 20), 'Displacement Calculation Settings' (with Initial Block Size of 32 px, Resolution Step of 16 px, Pixel Intensity Cut-off of 125, XCorrelation Threshold of 0.65, Max. Iteration Steps of 20, and Interpolation Method of 'v4'), and 'Gel Properties' (with Elastic Modulus of 20000 Pa and Poisson's Ratio of 0.48). Below these panels are 'Calculate Displacements' and 'Calculate Traction' buttons. On the right side, a 'Cell Boundary' panel contains an 'Outline Cell' button, which is highlighted with a red checkmark and a red mouse cursor arrow.

- There are two options for outlining the cell:
1. Draw an outline manually by holding left mouse button, dragging the cursor and double-clicking on the selected shape
 2. Utilize an automated cell detection algorithm

5. If cell detection filter was used click on a yellow painted cell ✓

The screenshot displays the TractionsForAll software interface. At the top, the 'File' menu is visible. Below it, the 'Current Folder' is set to 'D:\Experiments\test\01\'. A 'Load First Image' button is present. The main workspace is divided into several sections:

- Crop Images & Camera Settings:** Includes a 'Crop Images' button, ROI Size (px) set to 640, Pixel Size (um) set to 6.2111, and Objective Magnification set to 20.
- Displacement Calculation Settings:** Includes Initial Block Size (px) set to 32, Resolution Step (px) set to 16, Pixel Intensity Cut-off set to 125, XCorrelation Threshold set to 0.65, Max. Iteration Steps set to 20, and Interpolation Method set to 'v4'.
- Gel Properties:** Includes Elastic Modulus (Pa) set to 20000 and Poisson's Ratio set to 0.48.
- Analysis:** Includes 'Calculate Displacements' and 'Calculate Tractions' buttons.

The central image shows a grayscale image of a cell with a green rectangular ROI. Below it, the same image is shown with the cell highlighted in yellow. A red checkmark and a mouse cursor are positioned over the yellow cell. To the right, the 'Cell Boundary' section contains an 'Outline Cell' button and an 'Edge' button, with a red checkmark and mouse cursor over the 'Edge' button.

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6. Save cell outline by clicking on the 'Save' button ✓

The screenshot displays the TractionsForAll software interface. At the top, the 'File' menu is visible. Below it, the 'Current Folder' is set to 'D:\Experiments\test\01\'. A 'Load First Image' button is present. The main workspace is divided into two panels. The top panel shows a grayscale image of a cell with a green rectangular ROI. Above this image, the coordinates '[312 201 640 640]' are displayed. The bottom panel shows the same cell image with a red outline around its perimeter. To the left of the images are several settings panels: 'Crop Images & Camera Settings' (with 'Crop Images' button, ROI Size: 640 px, Pixel Size: 6.2111 um, Objective Magnification: 20), 'Displacement Calculation Settings' (with Initial Block Size: 32 px, Resolution Step: 16 px, Pixel Intensity Cut-off: 125, XCorrelation Threshold: 0.65, Max. Iteration Steps: 20, Interpolation Method: 'v4'), and 'Gel Properties' (with Elastic Modulus: 20000 Pa, Poisson's Ratio: 0.48). At the bottom left, there are 'Analysis' buttons for 'Calculate Displacements' and 'Calculate Tractions'. On the right side, a 'Cell Boundary' panel contains buttons for 'Outline Cell', 'Draw', 'Edge', and 'Save'. The 'Save' button is highlighted with a red checkmark and a red arrow pointing to it from the text below.

Note: If you want to analyze a time sequence of images you should outline all cell images separately. A click on 'Save' button will record all selected outlines.

7. Calculate displacement field for each image in the sequence ✓

The screenshot displays the TractionsForAll software interface. The main window shows a grayscale image of a cell with a green rectangular ROI. The ROI coordinates are [312 201 640 640]. The software is set to the current folder D:\Experiments\test\01\.

Crop Images & Camera Settings:

- ROI Size (px): 640
- Pixel Size (um): 6.2111
- Objective Magnification: 20

Displacement Calculation Settings:

- Initial Block Size (px): 32
- Resolution Step (px): 16
- Pixel Intensity Cut-off: 125
- XCorrelation Threshold: 0.65
- Max. Iteration Steps: 20
- Interpolation Method: 'v4'

Gel Properties:

- Elastic Modulus (Pa): 20000
- Poisson's Ratio: 0.48

Analysis:

- Buttons: Calculate Displacements (checked), Calculate Traction

Displacements Heatmap:

The heatmap shows displacement values in micrometers (μm) across the ROI. The x and y axes range from -80 to 80 μm. A color scale on the right indicates displacement values from 0.5 to 2.0 μm.

Annotations:

- A red arrow points from the Pixel Size (um) field to the heatmap, with the text: "Appropriate length scale will be determined by camera pixel size (in microns) and microscope objective magnification (ex. 20X)." and "Note: Check the technical documentation of the camera to find out what is the size of CCD sensor pixel."
- An orange arrow points from the Displacement Calculation Settings to a red box containing the text: "Parameters that will affect the calculation of displacement field." and "Note: For proper comparison, keep these parameters constant when analyzing an experiment (especially when the measurements are done on a range of gel stiffness conditions). Selected resolution will have large impact on calculation speed."

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8. Calculate corresponding tractions ✓

TractionsForAll

File

Current Folder: D:\Experiments\test\01\

Load First Image

[312 201 640 640]

Crop Images & Camera Settings

Crop Images

ROI Size (px) 640 Update

Pixel Size (um) 6.2111

Objective Magnification 20

Displacement Calculation Settings

Initial Block Size (px) 32

Resolution Step (px) 16

Pixel Intensity Cut-off 125

XCorrelation Threshold 0.65

Max. Iteration Steps 20

Interpolation Method v4

Gel Properties

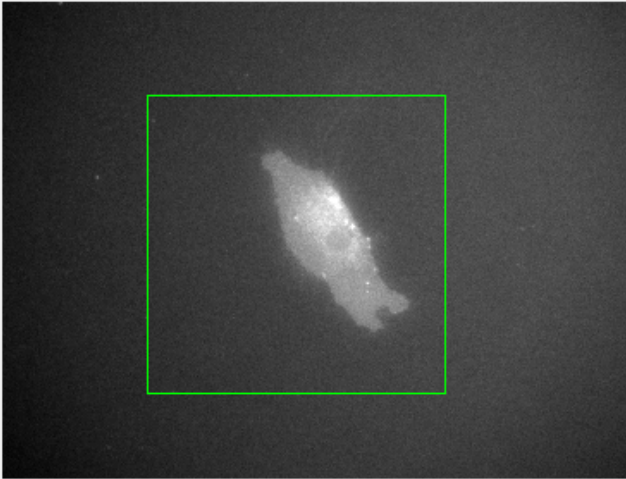
Elastic Modulus (Pa) 20000

Poisson's Ratio 0.48

Analysis

Calculate Displacements

Calculate Tractions ✓



Cell Boundary

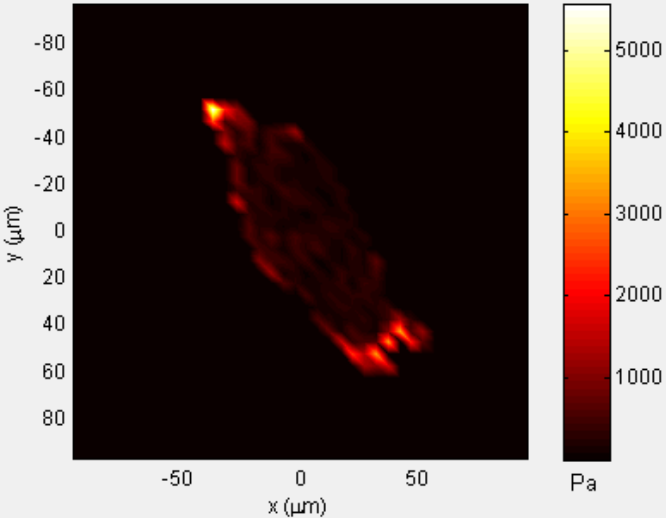
Outline Cell

Constrained t...		Principal mo...	
Contraction ...		Orientation o...	x*x* principal... y*
Mxx	Myy	Mxy	
-29.18749	-51.11876	-31.61397	54.56482 -73.61487 -6

Centroid coo...		Second mom...		Pr
x_bar (um)	y_bar (um)	bx_bar (um^4)	ly_bar (um^4)	bx_y_bar (um^4) Or
104.59617	101.84922	3642014.949...	1910537.609...	2005561.992... 56

Elastic properties of polyacrylamide gel will determine the magnitude of calculated tractions.

Tractions



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Pick a Map: Constrained Tractions

Export

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9. Edit and Export the plots ✓

TractionsForAll

File

Current Folder: D:\Experiments\test\01\

Load First Image

[312 201 640 640]

Crop Images & Camera Settings

Crop Images

ROI Size (px) 640 Update

Pixel Size (um) 6.2111

Objective Magnification 20

Displacement Calculation Settings

Initial Block Size (px) 32

Resolution Step (px) 16

Pixel Intensity Cut-off 125

XCorrelation Threshold 0.65

Max. Iteration Steps 20

Interpolation Method 'v4'

Gel Properties

Elastic Modulus (Pa) 20000

Poisson's Ratio 0.48

Analysis

Calculate Displacements

Calculate Tractions

All important output variables will be stored in an Excel file.

Constrained t...		Principal mo...		
Contraction ...		Orientation o...	x*x* principal... y*	
Mxx	Myy	Mxy		
-29.18749	-51.11876	-31.61397	54.56482 -73.61487 -6	
Centroid coo...		Second mom...		Pr
x_bar (um)	y_bar (um)	bx_bar (um^4)	ly_bar (um^4)	bx_bar (um^4) Or
104.59617	101.84922	3642014.949...	1910537.609...	2005561.992... 56

Tractions

Cell Boundary

Outline Cell

1/1

Pick a Map: Constrained Tractions

Export ✓

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Polyacrylamide Gel Preparation and Surface Conjugation of Fluorescent Microspheres

- Treat glass-bottom 24-well plates (In Vitro Scientific) with 0.4% aqueous solution of 3-methacryloxypropyltrimethoxysilane (Acros Organics) **at pH 3.5** for 1 h. Rinse three times in distilled water and air dry. Treated plates can stay on the shelf for months.
- Seven prepolymerization solutions of variable ratios of acrylamide:bisacrylamide (Bio-Rad) can be prepared in advance and stored at 4 °C [% acrylamide:% bisacrylamide (Young's modulus, kPa); 3:0.05 (0.3), 3:0.11 (1), 7.5:0.05 (6), 7.5:0.12 (13), 7.5:0.19 (17), 7.5:0.34 (20), 12:0.242 (75)] (see **Table 1** for detailed recipe; these solutions can be kept for weeks at 4 °C). Solutions could be sterilized by filtering through 0.22 µm filters.
- Prepare 1% ammonium persulfate (APS) aqueous solution (by mass, APS density is 1.98 g/ml) and 100 mM sodium bisulfite (SB) solution (SB MW ≈ 104 g/mol). These solutions can be mixed together in volume ratio 9 x APS:1 x SB and kept at 4 °C for several days – test it!). Solutions can be sterilized by filtering.
- Add ~20 µl of polymerization mixture (**ratio of prepolymerization solution:APS:SB = 90%:9%:1%**) in the well and sandwich it with SurfaSil-treated (Thermo Scientific), hydrophobic glass coverslips (15 mm in diameter for 24-well plate) **for 5 min**. The thickness of resulting gel will be ~100 µm.

- After polymerization, the gel surface can be derivatized with 1 mg/ml 3-Hydroxytyramine hydrochloride (Sigma) in 50 mM HEPES solution, **at pH 8.5-8.7 for 5 min (Important:** remove solution and wash the gels with water, or PBS, because oxidizing 3-Hydroxytyramine hydrochloride will become dark and it will be quickly absorbed in the gel).
- Fluorescent, 200 nm in diameter, sulfate-modified latex microspheres of chosen color (FluoSpheres, Invitrogen) will be conjugated to the gel surface when sonicated aqueous suspension of beads (diluted at 1:200) is delivered on top of the gels for 60 min. The gels should be rinsed three times in distilled water to remove all remaining nonattached beads.
- Gels should be UV sterilized for 1 h (microwaving the plate full of water for 30 sec, or until boiling point is reached, is an attractive alternative; **Be careful! Violent boiling can ruin your gels.**)
- Functionalize gels by incubation with 10 µg/ml of sterile collagen I (PureCol) in PBS for 2 h.

Note: THIS PROTOCOL DOESN'T REQUIRE ANY VACUUM DEGASSING OR POLYMERIZATION IN NITROGEN CHAMBER. POLYMERIZATION OCCURS IN 1-2 MINUTES, ON THE BENCH! PLAY WITH YOUR GEL SYSTEM! FIND OPTMAL POLYMERIZATION CONDITIONS FOR YOUR APPLICATION.

Table 1.**For 1 ml of gel solution:**

Measured E (Pa)	300	1000	6000	13000	17000	20000	75000
% Acrylamide	3	3	7.5	7.5	7.5	7.5	12
% Bisacrylamide	0.048	0.107	0.053	0.117	0.192	0.236	0.242

POLYMERIZATION MIXTURE (all in μ l) – PREPARED IN ADVANCE

40% Acrylamide	75	75	187.5	187.5	187.5	187.5	300
2% Bisacrylamide	24.5	53.5	27	58	88	118	120.5
Water	799	770	684	653	623	593	478
TEMED	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Total	900	900	900	900	900	900	900

Ammonium persulfate (APS) and sodium bisulfite (SB) solutions can be prepared in advance, but should be mixed together with **prepolymerization mixture just before gel casting**

1% APS (vol)	90	90	90	90	90	90	90
100 mM SB	10	10	10	10	10	10	10

For 50 ml of gel solution:

Measured E (Pa)	300	1000	6000	13000	17000	20000	75000
% Acrylamide	3	3	7.5	7.5	7.5	7.5	12
% Bisacrylamide	0.048	0.107	0.053	0.117	0.192	0.236	0.242

POLYMERIZATION MIXTURE (all in μ l) – PREPARED IN ADVANCE

40% Acrylamide	3750	3750	9375	9375	9375	9375	15000
2% Bisacrylamide	1225	2675	1350	2900	4400	5900	6025
Water	39950	38500	34200	32650	31150	29650	23900
TEMED	75	75	75	75	75	75	75
Total	45000	45000	45000	45000	45000	45000	45000

Ammonium persulfate (APS) and sodium bisulfite (SB) solutions can be prepared in advance, but should be mixed together with **prepolymerization mixture just before gel casting**

1% APS (vol)	4500	4500	4500	4500	4500	4500	4500
100 mM SB	500	500	500	500	500	500	500