

FM-Track

A fiducial marker tracking software for studying cell mechanics in a three-dimensional environment

Emma Lejeune^{1,2}, Alex Khang¹, Jacob Sansom^{1,3}, Michael S. Sacks^{1,†}

1. *James T. Willerson Center for Cardiovascular Modeling and Simulation, Oden Institute for Computational Engineering and Sciences and the Department of Biomedical Engineering, The University of Texas at Austin, Austin TX, United States.*

2. *The Department of Mechanical Engineering, Boston University, Boston MA, United States*

3. *The Department of Aerospace Engineering and Engineering Mechanics, The University of Texas at Austin, Austin TX, United States.*

[†] *Corresponding author.*

Abstract

Tracking the deformation of fiducial markers in the vicinity of living cells embedded in compliant synthetic or biological gels is a powerful means to study cell mechanics and mechanobiology in a three-dimensional environment. However, current approaches to track and quantify 3D fiducial marker displacements remain ad-hoc, can be difficult to implement, and may not produce reliable results. Herein, we present a compact software called “FM-Track,” written in the popular Python language, to facilitate feature-based particle tracking tailored for 3D cell micromechanical environment studies. FM-Track contains functions for pre-processing images, running fiducial marker tracking, and post-processing and visualization. FM-Track can thus aid researchers in cellular mechanics and mechanobiology by providing an extensible software platform to more reliably extract complex local 3D cell contractile information in transparent compliant gel systems.

Keywords:

1. Motivation and Significance

At present, there is substantial research focused on understanding how cells interact mechanically with their local environment. While the field

started with cells seeded on two-dimensional surfaces, [1, 2, 3, 4, 5], more emphasis is currently being placed on the higher realism of the three-dimensional cellular micro-environment where cells are embedded in transparent natural or synthetic gels [6, 7, 8, 9, 10, 11, 12]. One major class of methods for probing cell mechanics involves tracking the motion of fiducial markers in the vicinity of a given cell [13, 14]. For example, 3D Traction Force Microscopy (TFM) is an experimental technique that has gained substantial popularity in recent years [6, 7, 9, 10, 11, 15]. In TFM, cells and μm -scale fluorescent beads are seeded in gels. The cell is typically imaged, reagents to shut down the contractile machinery are delivered, and then the cell is imaged again. The observed deformation of the fluorescent beads in the vicinity of the cell is then used to infer the forces that the cell is imposing on its local environment given known mechanical properties of the gel [6]. However, widespread adoption of TFM, and related methods, is limited in part because the software required for successful bead tracking is not trivial to implement, and overall lacks standardization [13, 14, 16, 17, 18, 19, 20, 21]. This includes not only tracking but also features such as microscope translation correction and robust post-processing of the resulting displacement data. Such limitations make both the initial displacement measurement and subsequent interpretation difficult. Overall, there is a need for accessible software to enable cell mechanics research and promote standardized technique across different laboratories.

To address these issues, we have developed a software package entitled “FM-Track”, an open source software written entirely in the popular Python language. While there are multiple examples of feature based particle tracking algorithms described in the literature [6, 14], there are limited available open source software resources [18]. In the present work, we specifically build on other methods in the literature and introduce a simple open source particle tracking software written entirely in the Python language. Important novel aspects, to the author’s knowledge, are (1) tracking algorithm improvements such as our procedure for checking different permutations of feature matches and (2) a multivariate adaptive regression spline (MARS) based translation correction function that is necessary to overcome the potential microscope hardware limitations of unintended sample translation. In the following, we include a description of how FM-Track works in Section 2 and a minimum working example of running the algorithm presented in Section 3.

2. Software description

Many open source particle tracking algorithms are designed for systems with high time resolution, so that the change in particle positions between images is small [22]. Herein, we focus on systems where there are only two images, one initial image and one final image, with no specific limitation on the displacement magnitude. In this scenario, a successful algorithm must be able to accommodate large deformations between time frames, but computational efficiency is less critical given that the algorithm will only have to run once per image set and will never run in real time. Thus, the objective of this software is to go from two image stacks (e.g. acquired with confocal microscopy or an equivalent technique) of fluorescent beads and stained cells to a matched set of bead center positions.

This resulting process is broken down into three steps: pre-processing, tracking, and post-processing, and the software is written to easily accommodate different sets of input parameters. To operate the software, the user will feed variables (both file paths and tunable parameters) into the “InputInfo” class. The file “run_tracking_all_steps.py” is set up such that the user can run the algorithm on a set of images by calling the “run_tracking_all_steps()” function once.

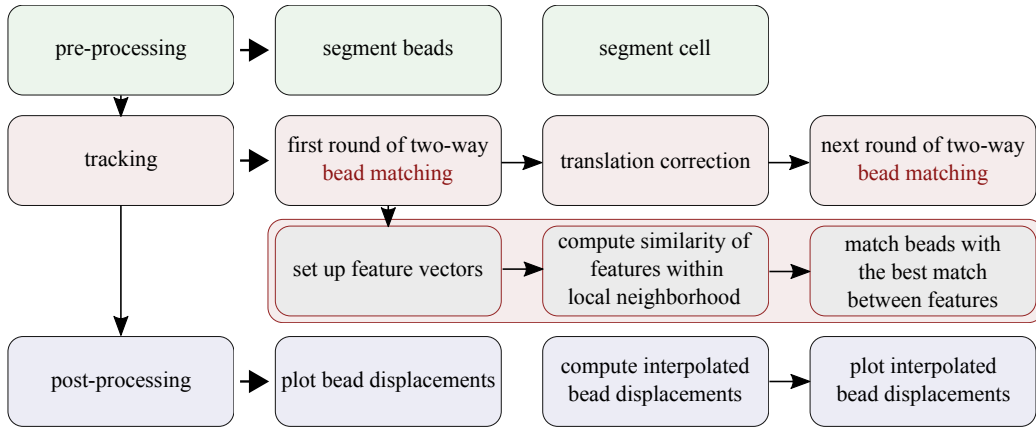


Figure 1: Basic flow chart of FM-Track.

2.1. Software architecture

Work flow in FM-Track is broken down into three types of functions: pre-processing, tracking, and post-processing. These functions are defined in

three separate files and are all called from the main function “`run_all_track()`”. User-specified input parameters and paths to image files are identified in a separate class “`InputInfo`”. The flow chart of FM-Track is illustrated in Fig. 1.

2.2. Software functionalities

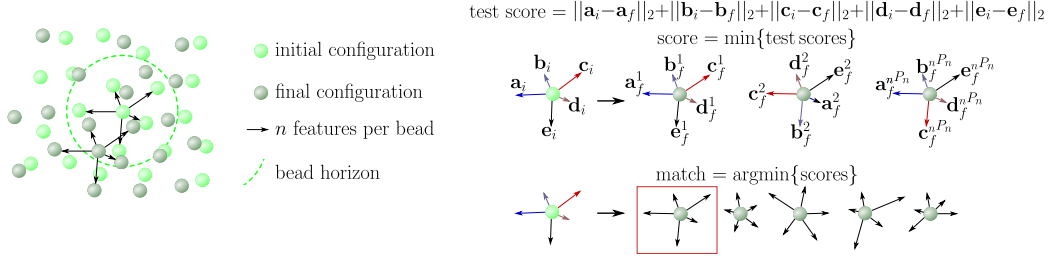


Figure 2: Schematic of the algorithm for feature based bead matching. The nP_n permutations of features for each bead within a defined horizon are compared, and the matched bead is the bead with the best score. Clashes are resolved by assigning the match to the pair with the better score. Two-way tracking means that the match must hold when the algorithm is run from initial configuration to final configuration and from the final configuration to the initial configuration.

The pre-processing file contains three functions. First, a function to import the three dimensional image stacks taken with a confocal microscope in the correct order as a `numpy` array. Second, a function to identify the coordinates of the center of every bead in a given image. Third, a function to identify the approximate volume, center, surface mesh, and surface mesh normals of a single cell in a given image. If there are multiple cells in the image, the function in its current state will only return the largest cell by volume.

The tracking file contains a main function “`track_main_call()`” to perform bead tracking given a specified first and second set of input images. If tracking type 1 is specified, the tracking function will run the tracking algorithm once. If tracking type 2 is specified, the tracking function will run the tracking algorithm, run a translation correction algorithm, and then run the tracking function again. The translation correction algorithm will correct for any translation that can be described as a function of z -position. A general outline of how the tracking function works is illustrated in Fig. 2. Once the tracking step is complete, the initial coordinates and displacement vector are recorded for every bead that was successfully matched. The tracking file

also contains a similar function called `“track_no_cell()”` that is suitable for running the algorithm when there is no cell present.

The post-processing functions are included for easy visualization of the bead tracking results. Several different types of plots of the raw bead displacements are available, as illustrated in Fig. 3 and Fig. 4. Furthermore, the post processing step also includes a function to interpolate the raw bead displacements using Gaussian Process Regression (GPR) `“create_GP_model”` and a function to produce filled color plots of the interpolations `“plot_gp_model”`. Further information on Gaussian Process Regression is available in the literature [23, 24].

3. Illustrative example

To illustrate the functionality of FM-Track, we present an example of a porcine Aortic Valve Interstitial Cell (AVIC) embedded in a PEG hydrogel containing suspended $0.5\ \mu\text{m}$ microspheres serving as fiducial markers [25]. Details of the methodology regarding AVIC encapsulation within a PEG hydrogel have been previously presented [25]. AVICS were imaged under normal conditions and imaged after it is treated with Cytochalasin-D, a chemical that inhibits actin polymerization and therefore shuts down the contractile machinery of the cell. By comparing the treated cell to the normal (untreated) cell, we are able visualize how the cell actively deforms the surrounding gel. A brief overview of the experimental protocol used to generate these images is included in Appendix 1. In Appendix 2, we also included two additional examples with synthetic data derived from a finite element model of a block undergoing uniaxial extension. The synthetic data is useful for evaluating the performance of our tracking algorithm. On synthetic data, our tracking algorithm performs quite well on the relevant bead densities up to realistic expected levels of deformation.

To run the code, first install FM-Track using the installation instructions specified in the `“README.md”` file located in the `“FM-Track”` repository. Next, move the sample data folder and the `“test.py”` script to the same directory. An example data set and the `“test.py”` script are both found in the examples folder of the repository. Finally, change the name of the `“root_directory”` variable at the top of `“test.py”` and run it using either a Python IDE or the terminal. Further instructions on how to run the script are found in the `“README.md”` file.

To implement the code on a new data set, it will be necessary to modify the following variables:

- “`root_directory`”: used to specify the path to the data folder
- “`filenames_cell`”: used to specify the path to all cell files (the images must be sequentially numbered, with 4 digits padded by zeros)
- “`filenames_beads`”: used to specify the path to all bead files
- “`savefnames`”: used to specify the unique filename that segmented cell and bead files will be saved as
- “`tracking_pairs`”: used to specify the tracking pairs, (these must match the unique filenames specified in “`savefnames`”)
- “`fov_dims`”: used to specify the microscope field of view dimensions

If necessary, changes can readily be made to: the specified fluorescent color channels in the RGB input images “`cell_channel`” and “`bead_channel`”; the threshold for segmenting the cell image “`cell_thresh`”; the tracking parameters “`num_feat`”, “`num_nearest`”, “`buffer_cell_tracking`”, and “`track_type`”; the translation correction parameters “`buffer_cell_translation`”; and the type(s) of figure to save post processed results as “`figtype_list`”.

Once the parameters are specified in `test.py`, we run the script. This file is shown in 3. The script calls the FM-Track function “`run_tracking_all_steps()`”, which runs the software on the specified data. The components of this function are as follows:

- Pre-process: get bead centers “`get_bead_centers()`”, get cell surface meshes “`get_cell_surface()`”
- Track: run the tracking step “`track_main_call()`”, the result of running this step are text files that contain the x , y , and z coordinates of each bead in the first configuration, and text files that contain the displacement of each bead u , v , and w
- Post-process: plot the raw data “`call_plot_main()`”, generate the Gaussian Process Regression (GPR) model to interpolate bead displacements “`create_GP_model()`”, plot the GPR model “`plot_gp_model()`” (note: generating the Gaussian Process model may be slow and the saved model may take up a large amount of file space)

```

1 root_directory = '/Users/<username>/Desktop/data'
2
3 # section (1)
4 # import the necessary modules to use FM-Track
5
6 from fmtrack.inputinfo import InputInfo
7 import numpy as np
8
9 # section (2)
10 # initialize all variables that will be passed into InputInfo
11
12 filenames_cell = [ \
13     'CytoD/Cell/Gel 2 CytoD%s.tif',\
14     'Endo1/Cell/Gel 2 Endo1%s.tif',\
15     'Normal/Cell/Gel 2 Normal%s.tif']
16 filenames_beads = [ \
17     'CytoD/Beads/Gel 2 CytoD%s.tif',\
18     'Endo1/Beads/Gel 2 Endo1%s.tif',\
19     'Normal/Beads/Gel 2 Normal%s.tif']
20
21 savefnames = [ \
22     'CytoDSave',\
23     'Endo1Save',\
24     'NormalSave']
25
26 tracking_pairs = [ \
27     ['CytoDSave', 'Endo1Save'],\
28     ['CytoDSave', 'NormalSave'],\
29     ['Endo1Save', 'NormalSave']]
30
31 fov_dims = np.array([149.95, 149.95, 140.0])
32
33 # section (3)
34 # create an InputInfo object
35
36 info = InputInfo(root_directory)
37 info.set_inputs(filenames_cell, filenames_beads, savefnames, tracking_pairs, fov_dims)
38
39 # section (4)
40 # additional, optional steps of the script
41
42 #out_folder_cell = 'Gel_cell_coords'
43 #out_folder_beads = 'Gel_bead_center_coords'
44 #out_folder = 'Post_proc_summary'
45 #info.set_out_folder_cell(out_folder_cell)
46 #info.set_out_folder_beads(out_folder_beads)
47 #info.set_out_folder(out_folder)
48
49 #info.cell_channel = 0 #CellBrite Red
50 #info.bead_channel = 1 #Green fluorescent beads
51 #info.cell_thresh = 1.0
52 #info.num_feat = 5
53 #info.num_nearest = 15
54 #info.buffer_cell_tracking = 0
55 #info.track_type = 2 # type 1 will NOT perform translation correction, type 2 will
56 #info.buffer_cell_translation = 30
57 #info.figtype_list = ['.png']
58 #info.plot_type = 6.0
59 #info.run_GP = False
60 #info.use_corrected_cell = True
61 #info.should_plot = True # toggles 3D plotting using PyVista
62 #info.print_progress = True # toggles all printing in algorithm
63
64 # section (5)
65 # run all steps of the program
66
67 info.run_tracking_all_steps(True,True,True)

```

Listing 1: Example implementation file.

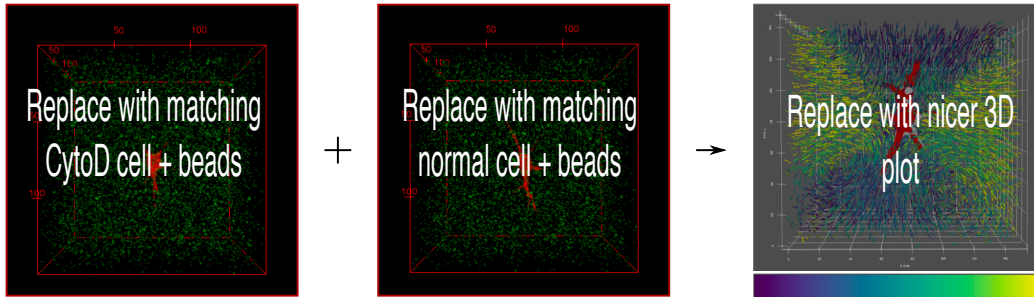


Figure 3: Left: 3D image stack of a cell (red) that has been chemically treated to suppress contractile machinery surrounded by fluorescent beads (green); Center: 3D image stack of a cell in a normal state surrounded by fluorescent beads; Right: superimposed image of both cells with bead displacements illustrated.

4. Impact and conclusions

The main benefit of FM-Track is that it provides an open source and simple to implement feature based bead tracking algorithm in the Python framework. Users have the option to implement the code directly as specified in the working example, use it with altered input parameters, or build on the functions and framework presented here in a manner that is entirely specific to their own needs. Important novel aspects of our code that are, to the author’s knowledge, not found elsewhere in the literature include: (1) minor aspects of the tracking algorithm (2) the MARS based translation correction function, and (3) an open source Python based framework. Our software is simple to use and modify, even for researchers with limited programming experience. Looking forward, we anticipate that FM-Track will enable research in Traction Force Microscopy and related techniques.

5. Acknowledgments

Funding was provided for this work by the Peter O’Donnell, Jr. Postdoctoral Fellowship awarded to Emma Lejeune, National Science Foundation Graduate Research Fellowship [Grant No. DGE-1610403] awarded to Alex Khang, and National Institutes of Health [Grant No. R01 HL-119297, HL-073021, and HL-142504] awarded to Michael S. Sacks.

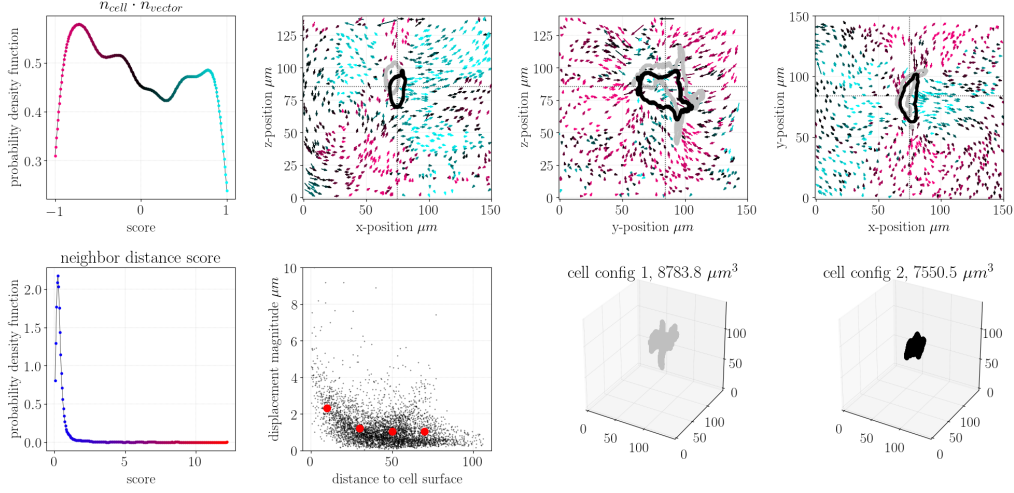


Figure 4: Summary of the results from FM-Track. Upper left: distribution of $n_{cell} \cdot n_{vector}$ where n_{cell} is the outward facing unit normal at the closest point on the cell surface and n_{vector} is the unit normal vector direction of bead motion; Upper right: projections of bead displacement through plane slices, where the gray outline is the cell in the initial configuration, the black outline is the cell in the final configuration, and bead displacements are color coded by direction compared to the cell surface; Lower left: comparison of bead displacements to their immediate neighbors (beads with high scores may be spurious); Lower center: bead displacement magnitude with respect to distance from cell surface; Lower right: three-dimensional plots of the cell in each configuration.

Appendix A. Experimental protocol used to generate the data used in Section 3

Appendix A.1. Sample preparation

Aortic heart valve leaflets were dissected from porcine hearts obtained from a local abattoir. Aortic valve interstitial cells (AVICs) were extracted from the leaflets via previously published methods [26]. AVICs were seeded at a density of 500,000 cells/mL along with $0.5 \mu m$ yellow-green fluorescent polystyrene microbeads (Polysciences) suspended at a density of 3×10^9 beads/mL within peptide-modified, poly (ethylene glycol) hydrogels comprised of MMP degradable peptide crosslinks (Bachem), CRGDS adhesive peptides (Bachem), 8-arm 40 *kDa* PEG-norbornene (Jenkem), lithium phenyl- 2,4,6 trimethylbenzophosphinate (LAP) photoinitiator (Sigma-aldrich), and PBS (Sigma-aldrich). The solution was pipetted into a petri dish with a 12 *mm* glass insert (Fisher Scientific) and polymerized underneath UV light (365

nm , $2.5 \text{ mW}/cm^2$) for 3 minutes. After that, the hydrogel construct was incubated at standard conditions in growth media for 72 hours before any experimentation was performed.

Appendix A.2. 3D Traction Force Microscopy Experiment

Image stacks ($150 \times 150 \times 140 \text{ } \mu m$ at $0.8 \text{ } \mu m$ z-spacing) of a single AVIC and the immediately surrounding fluorescent microbeads were captured using a Zeiss LSM 710 inverted confocal microscope with a 63x, 1.2 numerical aperture water-immersion objective lens (Zeiss). Two total image stacks were taken for every cell under normal and non-contractile conditions. For the normal case, the sample was incubated for 40 minutes within Trypdes Salt Solution (TSS, Sigma Aldrich) before the first image stack was acquired. After that, a non-contractile state was induced by adding a stock solution of Cytochalasin D (dissolved in DMSO, Sigma Aldrich) to achieve a final concentration of $4 \text{ } \mu M$. A second image stack of the same FOV was acquired after 40 minutes of incubation.

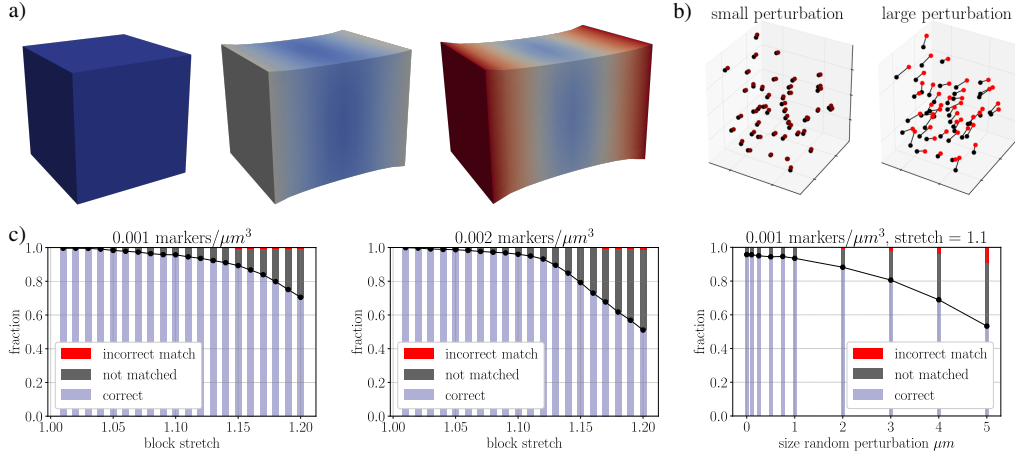


Figure A.1: a) illustration of a finite element model of a $100 \text{ } \mu m \times 100 \text{ } \mu m \times 100 \text{ } \mu m$ block under uniaxial displacement controlled extension; b) illustration of random perturbations to the bead positions, where the size of the random perturbation indicates the maximum perturbation in a single direction; c) summary of FM-Track performance on this synthetic data set.

Appendix B. Testing FM-Track performance on synthetic data

Appendix B.1. Note on synthetic data

Synthetic data is generated by randomly identifying points in the reference configuration of finite element simulations. The initial fiducial marker locations are the randomly identified points in the reference configuration, and the final fiducial marker locations are the randomly identified points in the current configuration after some boundary conditions or loading has been applied in the finite element setting. Notable, we shuffle the order of the fiducial markers in the final configuration in our synthetic data set.

Appendix B.2. Performance on synthetic data

Here we show the performance of our tracking algorithm on a synthetic data set. Fig. A.1a shows the finite element simulation used to generate the synthetic data, and Fig. A.1b shows the method for adjusting the synthetic data set to contain noise. In Fig. A.1c, we show the performance of FM-Track at two different marker densities, and on a data set with progressively higher levels of random perturbation. Notably, FM-Track performs quite well on block stretch up to 1.10, and with small random perturbations, and when it fails it tends to fail by not matching beads rather than by reporting incorrect matches.

- [1] H. Cirka, M. Monterosso, N. Diamantides, J. Favreau, Q. Wen, K. Billiar, Active traction force response to long-term cyclic stretch is dependent on cell pre-stress, *Biophysical Journal* 110 (8) (2016) 1845 – 1857. doi:<https://doi.org/10.1016/j.bpj.2016.02.036>.
URL <http://www.sciencedirect.com/science/article/pii/S0006349516300418>
- [2] R. W. Style, R. Boltyanskiy, G. K. German, C. Hyland, C. W. MacMinn, A. F. Mertz, L. A. Wilen, Y. Xu, E. R. Dufresne, Traction force microscopy in physics and biology, *Soft Matter* 10 (2014) 4047–4055. doi:[10.1039/C4SM00264D](https://doi.org/10.1039/C4SM00264D).
URL <http://dx.doi.org/10.1039/C4SM00264D>
- [3] M. Dembo, Y.-L. Wang, Stresses at the cell-to-substrate interface during locomotion of fibroblasts, *Biophysical Journal* 76 (4) (1999) 2307 – 2316. doi:[https://doi.org/10.1016/S0006-3495\(99\)77386-8](https://doi.org/10.1016/S0006-3495(99)77386-8).
URL <http://www.sciencedirect.com/science/article/pii/S0006349599773868>

- [4] J. P. Butler, I. M. Tolić-Nørrelykke, B. Fabry, J. J. Fredberg, Traction fields, moments, and strain energy that cells exert on their surroundings, *American Journal of Physiology-Cell Physiology* 282 (3) (2002) C595–C605. doi:10.1152/ajpcell.00270.2001.
- [5] J. L. Tan, J. Tien, D. M. Pirone, D. S. Gray, K. Bhadriraju, C. S. Chen, Cells lying on a bed of microneedles: An approach to isolate mechanical force, *Proceedings of the National Academy of Sciences* 100 (4) (2003) 1484–1489. doi:10.1073/pnas.0235407100.
- [6] W. R. Legant, J. S. Miller, B. L. Blakely, D. M. Cohen, G. M. Genin, C. S. Chen, Measurement of mechanical tractions exerted by cells in three-dimensional matrices, *Nature methods* 7 (12) (2010) 969.
- [7] M. S. Hall, R. Long, X. Feng, Y. Huang, C.-Y. Hui, M. Wu, Toward single cell traction microscopy within 3d collagen matrices, *Experimental cell research* 319 (16) (2013) 2396–2408.
- [8] K. Jaqaman, D. Loerke, M. Mettlen, H. Kuwata, S. Grinstein, S. L. Schmid, G. Danuser, Robust single-particle tracking in live-cell time-lapse sequences, *Nature methods* 5 (8) (2008) 695.
- [9] T. M. Koch, S. Münster, N. Bonakdar, J. P. Butler, B. Fabry, 3d traction forces in cancer cell invasion, *PloS one* 7 (3) (2012) e33476.
- [10] W. R. Legant, C. K. Choi, J. S. Miller, L. Shao, L. Gao, E. Betzig, C. S. Chen, Multidimensional traction force microscopy reveals out-of-plane rotational moments about focal adhesions, *Proceedings of the National Academy of Sciences* 110 (3) (2013) 881–886.
- [11] J. Steinwachs, C. Metzner, K. Skodzek, N. Lang, I. Thievessen, C. Mark, S. Münster, K. E. Aifantis, B. Fabry, Three-dimensional force microscopy of cells in biopolymer networks, *Nature methods* 13 (2) (2016) 171.
- [12] M. S. Hall, F. Alisafaei, E. Ban, X. Feng, C.-Y. Hui, V. B. Shenoy, M. Wu, Fibrous nonlinear elasticity enables positive mechanical feedback between cells and ecms, *Proceedings of the National Academy of Sciences* 113 (49) (2016) 14043–14048. arXiv:<https://www.pnas.org/content/113/49/14043.full.pdf>,

doi:10.1073/pnas.1613058113.

URL <https://www.pnas.org/content/113/49/14043>

- [13] M. K. Cheezum, W. F. Walker, W. H. Guilford, Quantitative comparison of algorithms for tracking single fluorescent particles, *Biophysical journal* 81 (4) (2001) 2378–2388.
- [14] X. Feng, M. S. Hall, M. Wu, C.-Y. Hui, An adaptive algorithm for tracking 3d bead displacements: application in biological experiments, *Measurement Science and Technology* 25 (5) (2014) 055701.
- [15] D. Song, N. Hugenberg, A. A. Oberai, Three-dimensional traction microscopy with a fiber-based constitutive model, *Computer Methods in Applied Mechanics and Engineering* 357 (2019) 112579.
- [16] N. Chenouard, I. Smal, F. De Chaumont, M. Maška, I. F. Sbalzarini, Y. Gong, J. Cardinale, C. Carthel, S. Coraluppi, M. Winter, A. Cohen, Objective comparison of particle tracking methods, *Nature methods* 11 (3) (2014) 281.
- [17] E. Meijering, O. Dzyubachyk, I. Smal, Methods for cell and particle tracking, in: *Methods in enzymology*, Vol. 504, Elsevier, 2012, pp. 183–200.
- [18] M. Patel, S. E. Leggett, A. K. Landauer, I. Y. Wong, C. Franck, Rapid, topology-based particle tracking for high-resolution measurements of large complex 3d motion fields, *Scientific reports* 8 (1) (2018) 5581.
- [19] S.-L. Liu, J. Li, Z.-L. Zhang, Z.-G. Wang, Z.-Q. Tian, G.-P. Wang, D.-W. Pang, Fast and high-accuracy localization for three-dimensional single-particle tracking, *Scientific reports* 3 (2013) 2462.
- [20] R. Boltyanskiy, J. W. Merrill, E. R. Dufresne, Tracking particles with large displacements using energy minimization, *Soft matter* 13 (11) (2017) 2201–2206.
- [21] F. Pereira, H. Stüer, E. C. Graff, M. Gharib, Two-frame 3d particle tracking, *Measurement science and technology* 17 (7) (2006) 1680.
- [22] J.-Y. Tinevez, N. Perry, J. Schindelin, G. M. Hoopes, G. D. Reynolds, E. Laplantine, S. Y. Bednarek, S. L. Shorte, K. W. Eliceiri, Trackmate:

An open and extensible platform for single-particle tracking, *Methods* 115 (2017) 80–90.

- [23] C. E. Rasmussen, Gaussian processes in machine learning, in: *Summer School on Machine Learning*, Springer, 2003, pp. 63–71.
- [24] F. Pedregosa, G. Varoquaux, A. Gramfort, V. Michel, B. Thirion, O. Grisel, M. Blondel, P. Prettenhofer, R. Weiss, V. Dubourg, J. Vanderplas, A. Passos, D. Cournapeau, M. Brucher, M. Perrot, E. Duchesnay, Scikit-learn: Machine learning in Python, *Journal of Machine Learning Research* 12 (2011) 2825–2830.
- [25] A. Khang, A. G. Rodriguez, M. E. Schroeder, J. Sansom, E. Lejeune, K. S. Anseth, M. S. Sacks, Quantifying heart valve interstitial cell contractile state using highly tunable poly(ethylene glycol) hydrogels, *Acta Biomaterialia* 96 (2019) 354 – 367. doi:<https://doi.org/10.1016/j.actbio.2019.07.010>. URL <http://www.sciencedirect.com/science/article/pii/S1742706119304908>
- [26] C. M. Johnson, M. N. Hanson, S. C. Helgeson, Porcine cardiac valvular subendothelial cells in culture: cell isolation and growth characteristics, *Journal of molecular and cellular cardiology* 19 (12) (1987) 1185–1193.