

Pearson *et al.* (2016)

Online Supplemental Material:

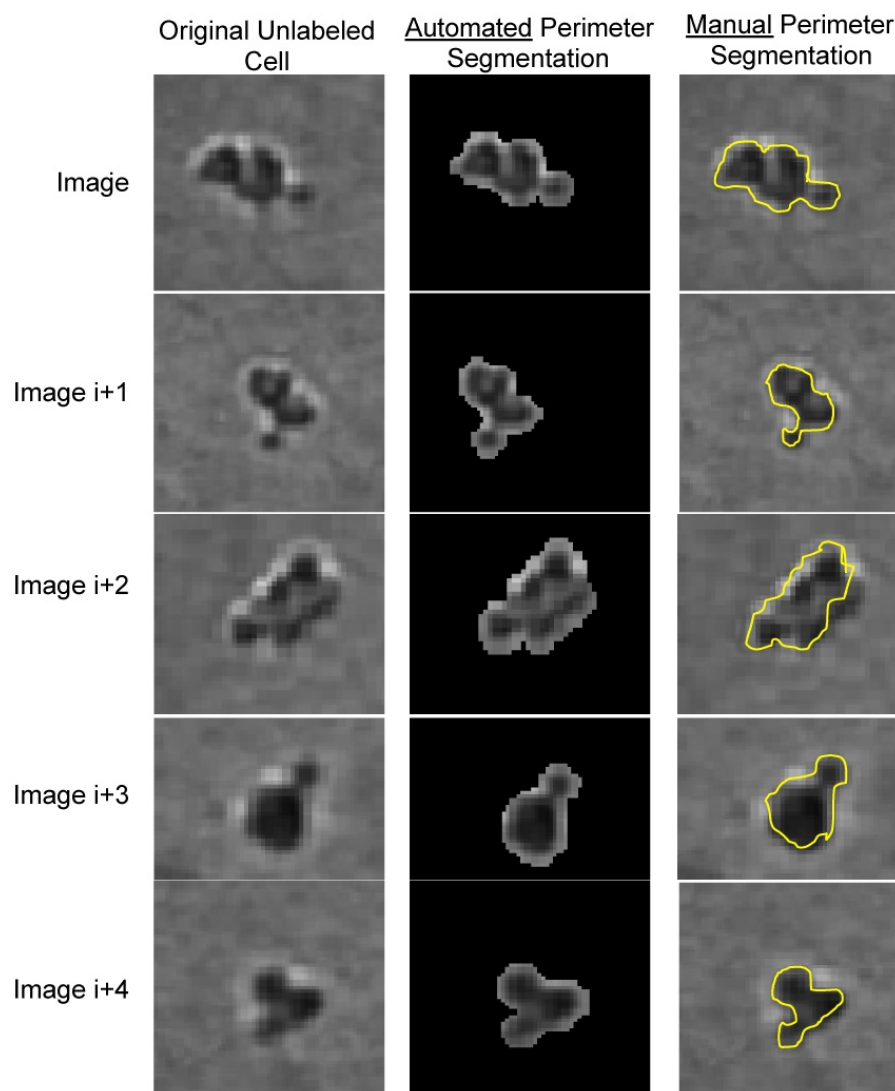


Figure S1: Manual and automated cell segmentation of representative bright field images of single cells. Cell migration is measured by approximating the center of mass via boundary pixel coordinates of each cell. Column 1 shows imaged T cells (including the grey scale pixel distribution of, and surrounding the cell). Column 2 shows a sample of auto-traced cells from CSPA. Column 3 shows a sample of cells with their manually traced-out perimeters. Notice (from row to row) the inconsistency in cell perimeter detection, as it is challenging (and temporally exhausting) to trace cells based on visually approximating grey-scale at the boundary. The consistency in auto-tracing based on grey-scale thresholding is key for (1) investigating morphological change and (2) identifying (pinpointing) regions of interest (ROI) for measuring biomechanical properties.

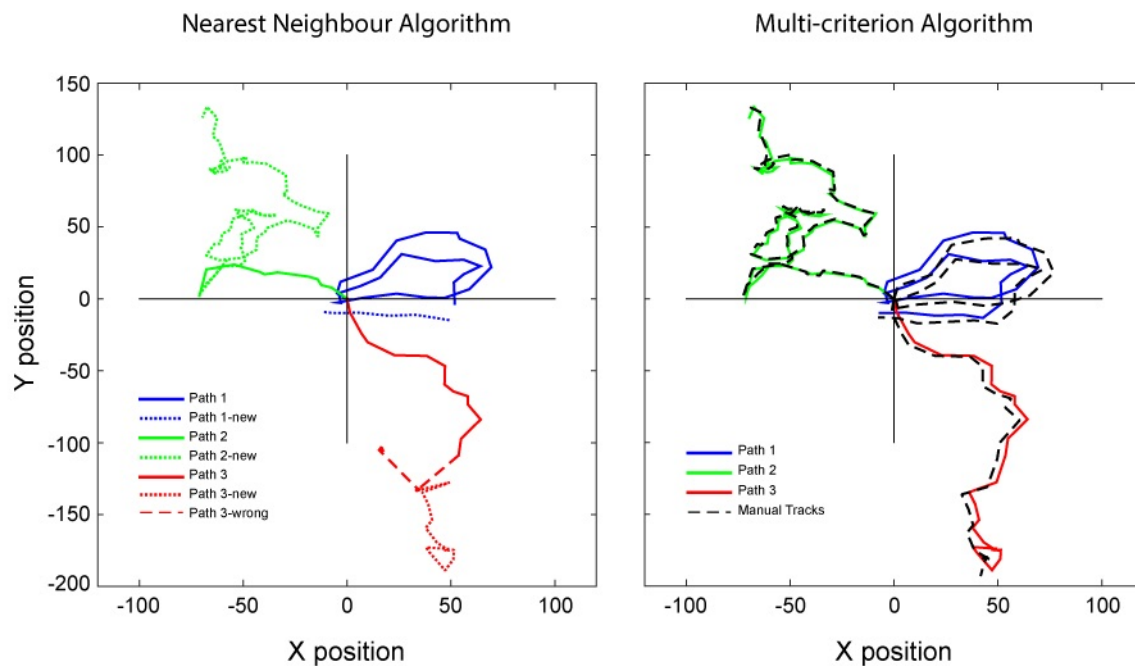


Figure S2. Comparison of T cell paths. **Left panel** shows a sub-sample of re-created tracks using MATLAB open source code *simple tracker* (Tinevez, 2012). This program takes as input centroid position coordinates from a ‘user-defined’ number of frames (images), by applying the nearest neighbor algorithm it links centroid positions to form estimated cell paths. When comparing to manually linked paths as seen in the **right panel** (-- black dash), the shortcoming of one basic criterion for path alignment is shown. For example, in **left panel** *Path #3 solid red* implies the cell was tracked accurately, dashed red (- - -) implies a new disjoint path was erroneously formed and (- - -) implies the cell path jumped tracks. **Right panel** Shows manual and automated cell paths. Automated paths were generated using the in-house segmentation and path alignment system we developed. We found that that the inclusion of additional path-linking criteria to be a fundamental step for achieving automated and accurate cell paths.

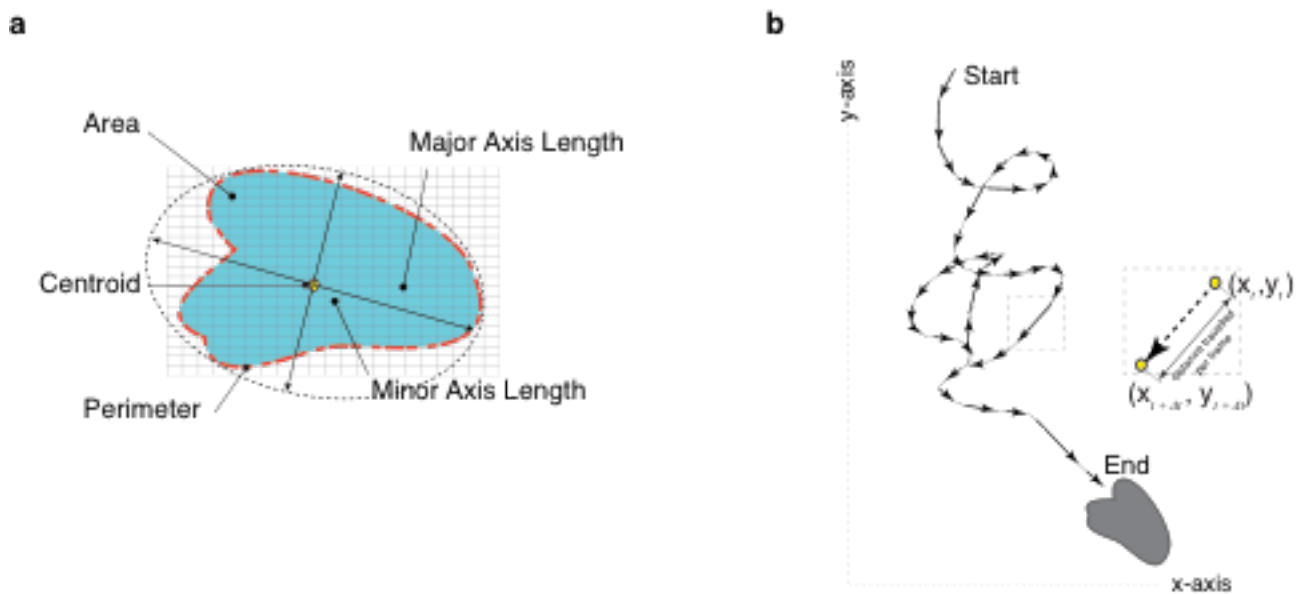


Figure S3. Extraction of morphological and migration parameters. (a) Morphological parameters are identified using segmentation outlines produced through CSPA, which isolate each cell. Cellular *Area* is measured as the sum of pixels falling within each region of interest (ROI, blue region) that represents a cell, *Perimeter* of each cell is measured as the pixel distance of the outlined ROI (dotted line), perimeter pixel coordinates are used as a reference for cell location and subsequent data retrieval. An ellipse is fitted within the ROI, which also aids in the calculation of major axis and minor axis lengths, their ratios defines the cellular aspect ratio (minor axis length / major axis length), whereby a number closer to unity defines a rounded or squamous cell and a ratio closer to zero indicates an elongated cell. (b) Lined up centroids produces migration paths of cells from which, migration parameters such as speed and mean squared displacement information are calculated. Parameters such as persistence, net distance, total distance, etc., can also be measured and used for further studies.

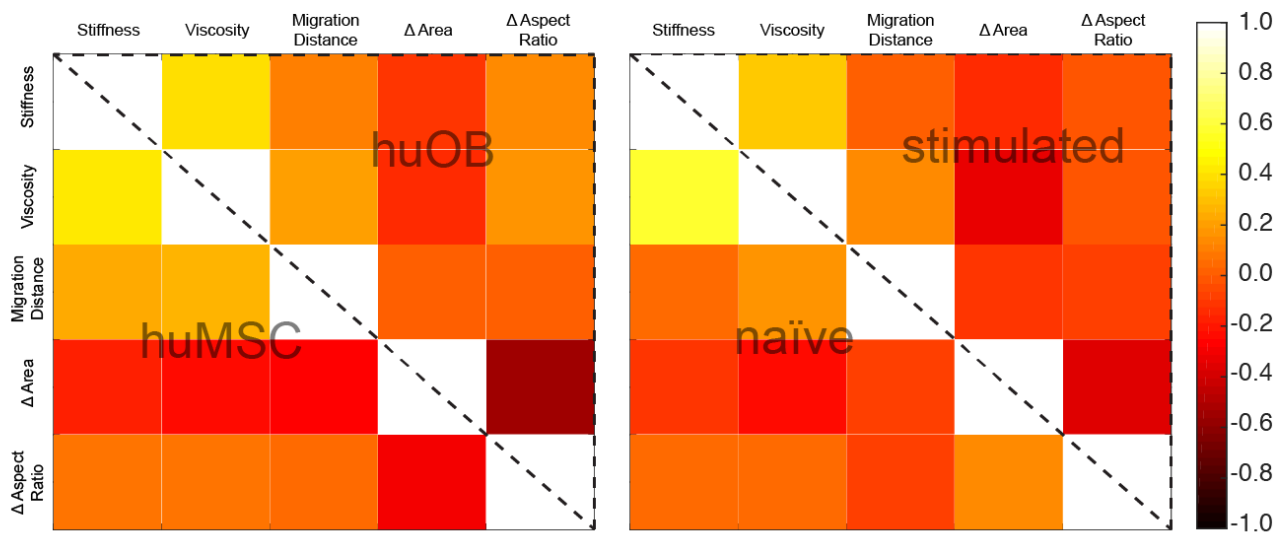


Figure S4. Correlation matrix between biomechanical, morphological and migration data from cell types quantified using the technique described by Pearson *et al.* (Left) Relationships between the different output parameters for human mesenchymal stem cells (huMSC) and huMSC after 5 days of osteoinduction (huOB). (Right) Comparison of relationships of naïve and stimulated T cells parameters. Color bar represents correlation coefficient (r).

Movie 1a



Movie 1b



Movie 1c



Movie 1d



Movie 1e



Movie 1f



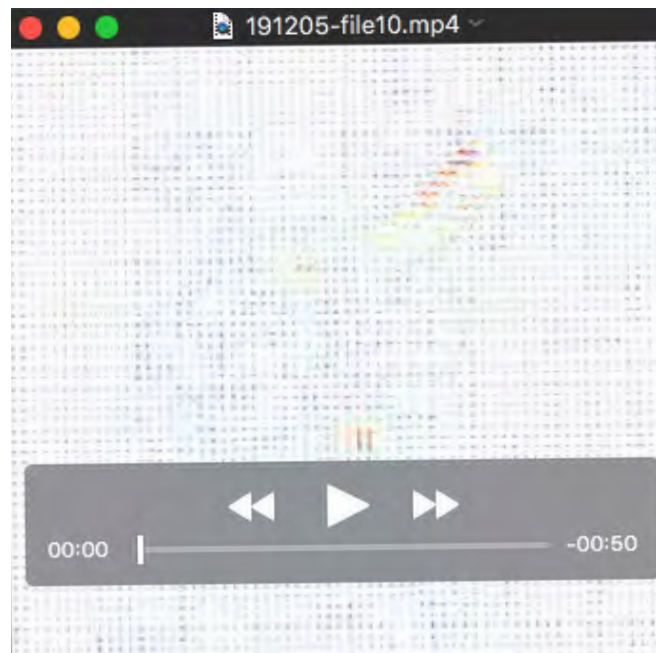
Movie 1. Comparison of calculated displacement and max principle strain fields of T-cells.

Displacement and maximum principle strain fields calculated from the image registration process of naive (a, b and c) and PMA/ionomycin stimulated (d, e, and f) T-cell datasets were overlaid onto the raw images as contour lines for visualization. It can be observed that naive T-cells have very fluid like movements through the 3D collagen matrix while activated T-cells move slower and have periods of dwelling time where they have long dendritic like extensions into the collagen matrix.

Movie 2a



Movie 2b



Movie 2c



Movie 2. Iterative changes during image morphing. (a) The image morphing algorithm iteratively changes the moving image (I_{t_n}) towards the fixed image ($I_{t_n+\Delta t}$) based on an image morphing algorithm, a non-rigid image registration process, whereby (b) the displacement field follows a Navier - Stokes model for viscoelastic flow that results in a displacement field (here represented as a quiver plot) that moves I_{t_n} . (c) Maximum principle strain information (here represented as a contour plot) is extracted per iteration, as I_{t_n} morphs into $I_{t_n+\Delta t}$, and used to approximate intrinsic cellular properties, stiffness and viscosity, based on Kelvin-Voigt model.

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Online Supplemental Tables:

Table S1. Comparison of automatic and manual mean squared displacement (MSD) and area values. Randomly selected cells (from naive T-cell datasets, $n = 32$, that range from 15 to 101 data time points, totaling 1401 centroid values) were manually tracked and segmented as a comparison against automated output using CSPA. Tables (a) and (b) show the absolute difference, and real percent difference between automated and manually measured MSD values. Tables (c) and (d) are the absolute and real percent difference between the automated and manually measured area.

		Mean	Stdev	Var	Mode	Min	Max
MSD	% Difference	-0.11	2.53	11.44	-6.83	-6.8.	5.47
	% Difference	2.22	2.04	8.10	0.13	0.13	8.82
Area	% Difference	25.89	33.37	1277.36	-7.89	-33.67	125.76
	% Difference	36.68	26.99	857.44	23.33	3.74	128.11

Table S2. Stiffness quantification of human mesenchymal stem cells (huMSC) and human osteoblasts (huOB) using atomic force microscopy (AFM). Investigators, albeit using a common technique in AFM, have shown a wide range of stiffness ratios (k_{huOB} / k_{huMSC}) when comparing measurements obtained from huMSCs and huOBs, 0.52 – 2.41, our label-free non-invasive algorithm measured a stiffness ratio of 0.97.

Cell Type	Methodology	Stiffness Results (kPa) mean, mean \pm SD or range	Stiffness ratio (k_{huOB} / k_{huMSC})	Reference
huMSC from bone marrow huOB from differentiation using osteogenic induction medium	Atomic force microscopy with Hertz model	huMSC: 4.3 huOB: 1.9	0.44	(Bongiorno et al., 2014)
huMSC from bone marrow huOB from commercial source	Atomic force microscopy with modified Hertz Model	huMSC: 1.5 – 2.5 huOB: 1.5 – 2.6	1.00	(Docheva et al., 2008)
huMSC from bone marrow huOB from differentiation using osteogenic induction medium	Atomic force microscopy with Hertz model	huMSC: 3.2 ± 1.4 huOB: 1.7 ± 1.0	0.53	(Titushkin and Cho, 2007)
huMSC from bone marrow huOB from differentiation using osteogenic induction medium	Atomic force microscopy with Thin layer Hertz model	huMSC: 3.2 ± 2.2 huOB: 6.5 ± 2.7	2.41	(Darling et al., 2008)
huMSC from bone marrow huOB from differentiation using osteogenic induction	Atomic force microscopy with Hertz model	N.A.	0.60	(Yourek et al., 2007)
huMSC from bone marrow huOB from differentiation using osteogenic induction	Non – invasive, label – free, computational approach	huMSC: $3.28 \pm 0.99 \times 10^{-7}$ huOB: $3.18 \pm 0.64 \times 10^{-7}$	0.97	This Study

Table S3. Comparison of clocked time of automated and manual segmentation and path alignment. Manual segmentation and tracking was performed by 3 separate individuals. Manual data collection requires, between 12 and 55 min per individual cell, automated data collection is expected to take no more than 14 seconds. On averaging manual collection will take 12.57 min with a standard deviation of 12.32 min, while automated averages around 7.20 seconds.

	Manual (min)	Automated (min)	% Difference
Total	465.25	4.46	-99.04
Mean (min)	12.57	0.12	-99.04
Stdev (min)	12.32	0.06	-99.54
Min.	1.50	0.05	-96.52
Max.	55.20	0.23	-99.58