Barry et al., http://www.jcb.org/cgi/content/full/jcb.201501081/DC1

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1:  for every frame, f do
2:    for every frame, k, where f ≤ k ≤ f + n do
3:      for each candidate point, P, in frame k do
4:        if the current candidate has not already been assigned to a trajectory then
5:          if k == f then
6:            T = {P} //initialise a new trajectory, T, with P
7:            T → s_temp = ∞
8:          else
9:            i_min = −1, s_min = ∞
10:           for each existing trajectory, T do //P_E = {x_E, y_E, t_E, c_E} was the last point added to T
11:             if t_E == f and k > f then
12:               s = ||P_E − P||
13:               if s < s_min then
14:                 s_min = s, i_min = T → i //i denotes the index of T
15:           if i_min > −1 and s_min < n and s_min < T_{i_min} → s_temp then
16:             T_{i_min} → s_temp = s_min
17:             T_{i_min} → P_temp = P //T is noted as the current "winning" trajectory and P is temporarily associated with it
18:   for each existing trajectory, T do
19:     if T → s_temp < ∞ then
20:       if T → P_temp → t ≤ m + 1 then
21:         permanently assign P_temp to T and remove P_temp from the list of candidates
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Figure S1. **Algorithm for tracking anchor points.** A list of candidate points is compiled, each of which must meet the user-specified criteria for curvature threshold and curvature window (Fig. 5 B). Each candidate, P, has an associated set of coordinates, (x_P, y_P, t_P), and curvature value, c_P. Trajectories are constructed using an approach similar to that described previously (Sbalzarini and Koumoutsakos, 2005).

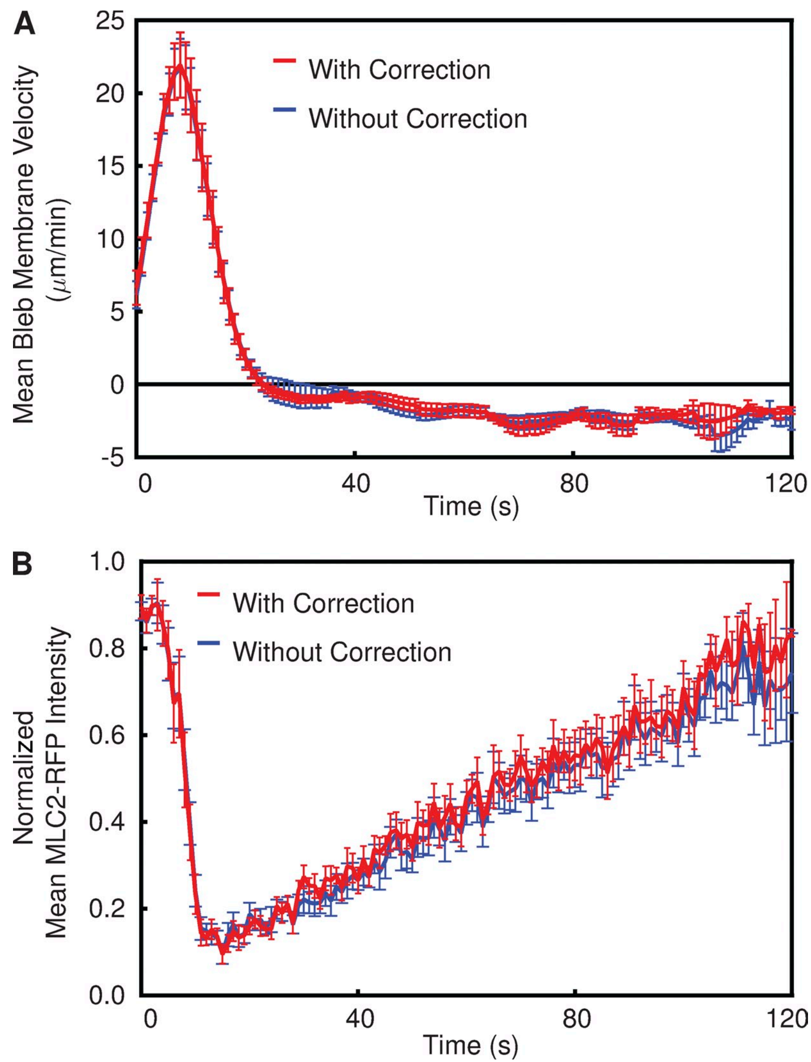
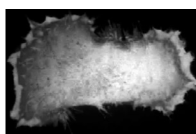


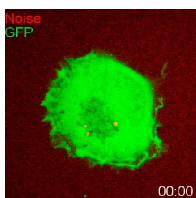
Figure S2. **Influence of manually excluding inaccurate bleb detections.** (A) Mean velocity profiles for corrected and uncorrected data, corresponding to the analysis output in Video 6, are virtually indistinguishable. (B) No statistically significant difference was found between the rates of MLC2-RFP fluorescence intensity increase for corrected and uncorrected data. Fitting a straight line to the linear portions of the plots (from 20 to 100 s) gave 95% confidence intervals for slope of 6.16–6.88 × 10^{-3} and 5.92–6.63 × 10^{-3} for corrected and uncorrected data, respectively. Error bars represent standard error of the mean.

Table S1. **Randomly generated ADAPT input parameter values used to generate the data in Fig. 7 E**

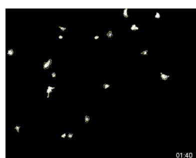
Spatial filter radius	Temporal filter radius	Curvature window	Minimum curvature threshold	Cortex depth	Signal threshold factor	Signal map threshold
μm	*s*			*μm*		
4.043	4.841	4	0.922	0.498	0.024	0.209
5.281	4.348	4	1.830	0.505	0.272	0.203
5.910	4.244	6	3.796	0.720	0.461	0.220
5.484	5.585	5	0.786	0.663	0.086	0.209
4.739	4.099	5	3.896	0.552	0.153	0.246
5.484	4.567	5	0.520	0.498	0.317	0.204
4.532	4.227	6	2.275	0.527	0.038	0.292
5.258	5.159	4	1.428	0.703	0.495	0.252
5.235	5.155	4	3.805	0.585	0.126	0.222
4.631	4.148	5	3.514	0.625	0.375	0.292
4.082	4.060	4	3.740	0.597	0.052	0.259
4.854	5.620	4	0.154	0.704	0.460	0.217
5.959	4.599	4	2.784	0.701	0.398	0.239
4.542	4.173	5	1.296	0.703	0.170	0.226
5.148	4.719	4	1.608	0.585	0.371	0.273
5.479	4.421	5	1.251	0.662	0.406	0.267
4.538	4.514	4	1.491	0.616	0.034	0.281
5.272	4.134	6	3.206	0.699	0.159	0.209
5.760	4.938	5	4.891	0.666	0.077	0.298
5.057	5.677	4	3.663	0.653	0.473	0.273



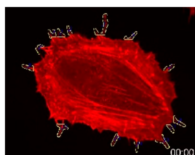
Video 1. **Whole-cell analysis using ADAPT.** (left) Time-lapse video of a HeLa cell expressing mCherry. Cell segmentation, calculated based on the mCherry signal (see Fig. 1, B and C), is shown on the right—green depicts expansion, and red shows retraction. The morphodynamic data derived from the analysis are shown in Fig. 1. Images were acquired with a 63× objective on a custom-built spinning-disc confocal microscope (Intelligent Imaging Innovations) at a frame rate of six frames per minute for 1 h. Time is shown in minutes and seconds. Image dimensions are 108 × 108 μm.



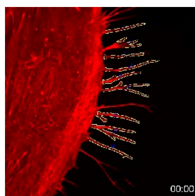
Video 2. **Correlation of H1080 cell morphodynamics with fluorescence intensities.** HT1080 cells expressing mCherry and GFP-Abi1 (left), GFP stained with CellMask Deep Orange (center), and GFP only (right). Images were acquired with a 63× objective on a custom-built spinning-disc confocal microscope (Intelligent Imaging Innovations) at a frame rate of two frames per minute for 40 min. Time is shown in minutes and seconds. Image dimensions are 73 × 73 μm.



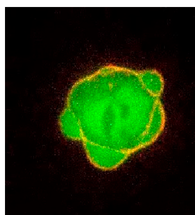
Video 3. **Tracking of HT1080 cell migration with ADAPT.** Time-lapse video of HT1080 cells stably expressing GFP. Cell segmentations are overlaid on the original images—green depicts expansion, and red shows retraction. Images were acquired in an IncuCyte FLR (Essen Bioscience) at a frame rate of 0.2 frames per minute for 4.5 h. Time is shown in hours and minutes. Image dimensions are 847 × 684 μm.



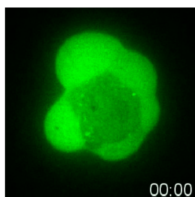
Video 4. **Tracking filopodia on a HeLa cell.** A HeLa cell stably expressing lifeact-GFP (red) with filopodia detected by ADAPT outlined in yellow. Images were acquired with a 63× objective on a custom-built spinning-disc confocal microscope (Intelligent Imaging Innovations) at a frame rate of two frames per minute for 25 min. Time is shown in minutes and seconds. Image dimensions are 108 × 85 µm.



Video 5. **High-resolution tracking of filopodia on a HeLa cell.** A HeLa cell stably expressing lifeact-GFP (red) with filopodia detected by ADAPT outlined in yellow. Images were acquired with a 100× objective on a custom-built spinning-disc confocal microscope (Intelligent Imaging Innovations) at a frame rate of one frame per second for 50 s. Time is shown in minutes and seconds. Image dimensions are 68 × 68 µm.



Video 6. **ADAPT analysis of a vaccinia-infected HeLa cell.** A HeLa cell expressing GFP (green) and MLC2-RFP (red) approximately 3 h after infection with vaccinia virus. Each bleb detected by ADAPT is highlighted and assigned an index number (left). The morphodynamic data associated with each detected bleb, together with temporal changes in MLC2 localization, are output to a text file in a directory specified by the user. The video allows the user to validate the results produced by the software. Images were acquired with a 63× objective on a custom-built spinning-disc confocal microscope (Intelligent Imaging Innovations) at a frame rate of one frame per second for ~8 min. Time is shown in minutes and seconds. Image dimensions are 58 × 58 µm.



Video 7. **Visualization of ArpC2-GFP in a blebbing HeLa cell.** A HeLa cell stably expressing ArpC2-GFP ~4 h after infection with vaccinia virus. Images were acquired with a 63× objective on a custom-built spinning-disc confocal microscope (Intelligent Imaging Innovations) at a frame rate of one frame per second for 5 min. Time is shown in minutes and seconds. Image dimensions are 59 × 59 µm.

A ZIP file is also provided that includes the JAR files necessary to install and run ADAPT, the source code is packaged within the JAR files, and test data are also included.

Reference

Sbalzarini, I.F., and P. Koumoutsakos. 2005. Feature point tracking and trajectory analysis for video imaging in cell biology. *J. Struct. Biol.* 151:182–195. http://dx.doi.org/10.1016/j.jsb.2005.06.002