

1 Robust cell tracking in epithelial tissues through identification 2 of maximum common subgraphs

3 Jochen Kursawe^{1,*}, Rémi Bardenet², Jeremiah J. Zartman³, Ruth E. Baker¹, Alexander G.
4 Fletcher^{4,5,*}

5 ¹ Mathematical Institute, University of Oxford, Andrew Wiles Building, Radcliffe Observatory
6 Quarter, Woodstock Road, Oxford, OX2 6GG, UK

7 ² CNRS & CRISAL, Université de Lille, 59651 Villeneuve d'Ascq, France

8 ³ Department of Chemical and Biomolecular Engineering, University of Notre Dame, 205D
9 McCourtney Hall of Molecular Science and Engineering, Notre Dame, IN 46556, USA

10 ⁴ School of Mathematics and Statistics, University of Sheffield, Hicks Building, Hounsfield
11 Road, Sheffield, S3 7RH, UK

12 ⁵ Bateson Centre, University of Sheffield, Sheffield, S10 2TN, UK

13 * E-mail: kursawe@maths.ox.ac.uk, a.g.fletcher@sheffield.ac.uk

14 **Data accessibility:** The datasets and code supporting this article are publicly available at
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20 data. All authors gave final approval for publication.

21 **Keywords:** Cell tracking, planar graphs, maximum common subgraph, epithelial sheets

22 **Abstract**

23 Tracking of cells in live-imaging microscopy videos of epithelial sheets is a powerful tool for in-
24 vestigating fundamental processes in embryonic development. Characterising cell growth, pro-
25 liferation, intercalation and apoptosis in epithelia helps us to understand how morphogenetic
26 processes such as tissue invagination and extension are locally regulated and controlled. Accu-
27 rate cell tracking requires correctly resolving cells entering or leaving the field of view between
28 frames, cell neighbour exchanges, cell removals and cell divisions. However, current tracking
29 methods for epithelial sheets are not robust to large morphogenetic deformations and require
30 significant manual interventions. Here, we present a novel algorithm for epithelial cell tracking,
31 exploiting the graph-theoretic concept of a ‘maximum common subgraph’ to track cells between
32 frames of a video. Our algorithm does not require the adjustment of tissue-specific parameters,
33 and scales in sub-quadratic time with tissue size. It does not rely on precise positional informa-
34 tion, permitting large cell movements between frames and enabling tracking in datasets acquired
35 at low temporal resolution due to experimental constraints such as phototoxicity. To demonstrate
36 the method, we perform tracking on the *Drosophila* embryonic epidermis and compare cell-cell
37 rearrangements to previous studies in other tissues. Our implementation is open source and
38 generally applicable to epithelial tissues.

39 1 Introduction

40 Live-imaging microscopy is a powerful, and increasingly quantitative, tool for gaining insight into
41 fundamental processes during embryonic development [1–3]. Quantitative information on cell
42 growth, proliferation, death, shape changes and movement extracted from live-imaging reveals
43 how such processes are regulated to give correct tissue-level behaviour. This approach has
44 been particularly successful in characterising the growth and patterning of embryonic epithelial
45 tissues in a number of model organisms [4–9].

46 A common experimental technique for visualising cell shapes in an epithelial sheet is to flu-
47 orescently tag a molecule marking cell boundaries, such as E-cadherin (figure 1A). The analysis
48 of time-lapse microscopy data obtained from such tissues is extremely challenging [2, 3], espe-
49 cially in cases of imaging data of rapidly evolving tissues, and when limitations of, for example,
50 microscope speed, imaging resolution or phototoxicity prohibit the creation of datasets with
51 high temporal and spatial resolution.

52 The analysis of time-lapse microscopy data comprises two major steps: segmentation and
53 tracking (registration). Segmentation must be performed for each frame of a video and in-
54 volves the identification of objects and landmarks, such as cell shapes (figure 1B). Automated
55 segmentation is hindered by various factors such as noise in fluorescent signals, uneven illu-
56 mination of the sample, or overlapping cells in a two-dimensional projection. Often, manual
57 correction is necessary to address over-segmentation, where too many cells are detected, or
58 under-segmentation, where too few cells are detected [10–12]. Tracking involves the association
59 of segmented cells across video frames (figure 1C) and requires resolving cellular movement, cell
60 division, cell death, and cells entering and leaving the field of view [12].

61 Numerous algorithms are available for the segmentation and tracking of cellular-resolution
62 microscopy data [10, 11, 13]. Common methods for cell tracking utilize optimization techniques
63 to minimise differences in cellular properties between two frames [11, 14–17]. The min-cost
64 max-flow algorithm [14] uses linear integer programming to minimise differences in cell areas,
65 perimeters, orientations, and locations between frames, whereas multiple-parameter tracking
66 [15] employs global optimization to minimize differences in cell shapes as well as locations.
67 In contrast, multitemporal association tracking [16, 17] minimises differences in cell locations
68 and sizes by using a probabilistic approach that finds the most likely extension to existing cell

69 trajectories. Chain-graph models [18] minimise differences in cell velocity while overcoming mis-
70 segmentation by verifying that each segmented object continues or begins a cell trajectory in
71 successive frames. Optical flow (‘warping’) between successive frames can be used to guide cell
72 tracking as well as segmentation [19]. It is also possible to combine segmentation and tracking
73 of 2D microscopy videos by interpreting time as a third spatial dimension and employing 3D
74 segmentation techniques [20]. The nearest-neighbour method associates two cells in consecutive
75 frames with each other if their respective centroids have minimal distance within the field of
76 view [10], or if their overlap in pixels within the field of view is maximal [21,22]. Particle image
77 velocimetry, a technique originally developed to analyse fluid flow [23], has also been employed
78 to track cells in epithelial tissues [24].

79 Software implementations and computational tools for cell tracking include FARSIGHT [25]
80 (segmentation only), SeedWaterSegmenter [10] (nearest-neighbour tracking), ilastik [18] (chain-
81 graph models), Tufts Tissue Tracker [11] (min-cost max-flow algorithm), Tracking with Gaus-
82 sian Mixture Models [26] (nearest-neighbour tracking), Packing Analyzer [27] (particle image
83 velocimetry) and EpiTools [13] (nearest-neighbour tracking). These algorithms and software
84 tools primarily rely on there being small differences in cell positions and shapes across consecu-
85 tive images. Their performance is therefore hindered when analysing data from *in vivo* studies
86 where phototoxicity provides a barrier to high temporal resolution imaging [28–30]. To address
87 this limitation, we propose a novel algorithm for cell tracking that uses only the connectivity of
88 cell apical surfaces (figure 1). By representing the cell sheet as a physical network in which each
89 pair of adjacent cells shares an edge, we show that cells can be tracked between successive frames
90 by finding the *maximum common subgraph* (MCS) of the two networks: the largest network of
91 connected cells that is contained in these two consecutive frames. It is then possible to track
92 any remaining cells based on their adjacency to cells tracked using the MCS. Our algorithm
93 does not require the tuning of parameters to a specific application, and scales in subquadratic
94 time with the number of cells in the sheet, making it amenable to the analysis of large tissues.

95 We demonstrate here that our algorithm resolves tissue movements, cell neighbour ex-
96 changes, cell division, and cell removal (for example, by delamination, extrusion, or death)
97 in a large number of *in silico* datasets, and successfully tracks cells across sample segmented
98 frames from *in vivo* microscopy data of a stage-11 *Drosophila* embryo. We further show how

99 our algorithm may be used to gain insight into tissue homeostasis by measuring, for example,
100 the rate of cell rearrangement in the tissue. In particular, we find a large amount of cell rear-
101 rangement within the observed dataset despite the absence of gross morphogenetic movement.
102 The remainder of the paper is structured as follows. In Section 2 we describe the algorithm
103 for cell tracking. In Section 3 we analyse the performance of the algorithm on *in silico* and *in*
104 *vivo* datasets. Finally, in Section 4 we discuss future extensions and potential applications.

105 2 Methods

106 In this section we provide a conceptual overview of the core principles underlying our cell
107 tracking algorithm. We focus on providing an accessible, non-technical description rather than
108 including all details required to implement the algorithm from scratch. A comprehensive math-
109 ematical description of the algorithm is provided in Supplementary Material S1.

110 The input to the algorithm is a set of segmented images obtained from a live-imaging
111 microscopy dataset of the apical surface of an epithelial cell sheet. For each image, the seg-
112 mentation is assumed to have correctly identified which cells are adjacent and the locations of
113 junctions where three or more cells meet. Various publicly available segmentation tools can be
114 used for this segmentation step, for example SeedWaterSegmenter [10] or ilastik [18]. The seg-
115 mentation is used to generate a polygonal approximation to the cell tessellation (figure 1B-C).
116 Such approximations are an adequate assumption for many epithelia [11, 31–34].

117 Our algorithm tracks cells by interpreting the polygonal representations arising from the
118 segmentation as networks (‘graphs’) of cells. Examples of such networks are shown in figure 2A.
119 In this representation, each cell corresponds to a vertex of the network, and two vertices are
120 connected by an edge if the corresponding cells are adjacent. Our algorithm tracks cells across
121 consecutive images by aligning the networks of cells that correspond to these images. This
122 network alignment is achieved in three steps. First, we generate an initial tracking for subsets
123 of the cells in each pair of consecutive images by finding the MCS between the two corresponding
124 networks (figure 2B). Second, this MCS is reduced to avoid tracking errors (figure 2C). Third,
125 remaining untracked cells are tracked based on their adjacency to cells within the MCS (figure
126 2D). In the final output of the algorithm, each tracked cell of a frame is paired with exactly one
127 cell in the subsequent frame.

128 The key step in this network alignment approach is the identification of a MCS [35,36]. A
129 MCS comprises the largest sub-network that is contained in two larger networks; thus finding
130 an MCS can be understood as recognising patterns of connections that are preserved between
131 two networks. In the present work, the structure of the MCS roughly corresponds to cells that
132 do not rearrange between consecutive images, except for few cells at its boundaries.

133 In figure 2B, we visualize the MCS generated by our algorithm as a collection of green
134 (light) and purple (dark) cells. Most of the highlighted cells in figure 2B are tracked correctly
135 by the MCS. Three cells in each frame are marked by a yellow (bright) dot. Within the two cell
136 networks, these cells are members of the MCS. However, these cells are not tracked correctly
137 by the MCS. This mismatch arises since the MCS is found based on the connectivity of cells
138 within the network alone. The fewer connections a cell has to other cells in the MCS, the less
139 information about the cell's position and shape is encoded by these network connections, and
140 so the greater the possibility of mismatches. To avoid such tracking errors, we remove any cells
141 that have only a few connections within the MCS, as well as small isolated clusters of cells.
142 All cells that are removed from the tracking in the second step of our algorithm are shown in
143 purple (dark) in figure 2B. In figure 2C we highlight the cells that are tracked after applying
144 this second step of our algorithm.

145 Cells that are untracked after reducing the MCS are then tracked based on their connections
146 to previously tracked cells. This last step of our algorithm comprises starting from the MCS
147 and iteratively 'growing' the set of tracked cells by adding cell-to-cell matches to the tracking
148 that maximise the number of preserved connections to other tracked cells. In this step, the
149 algorithm also resolves cell neighbour exchanges, cell removals, and cell divisions by identifying
150 changes in the network structure that are characteristic of these events. For example, a cell
151 neighbour exchange corresponds to the deletion of a network connection while a new connection
152 is added.

153 **The MCS is identified through repeated seeding and iterative extension** In computer
154 science, MCS finding has been known to be an NP-hard problem [35,36]: the time to find an
155 exact MCS of two networks increases exponentially with the size of the networks, which poses
156 a computational barrier to the use of MCS-finding algorithms in applications. We overcome
157 this computational barrier in the present work by constructing the MCS iteratively from the

158 MCSs of smaller subgraphs, exploiting the planar structure of our cell networks to reduce the
159 complexity of the problem.

160 To start the construction of the MCS, the algorithm identifies a match between two cells
161 in the consecutive images for which the structure of the network of their surrounding cells
162 is identical. Here, the network of surrounding cells is restricted to the network formed by a
163 cell's neighbours and its second nearest neighbours (see figure 3B). If no such initial match
164 can be found, the algorithm instead searches for an initial match where only the first-order
165 neighbourhood is preserved, under the condition that this neighbourhood does not touch the
166 boundary of the tissue. This latter condition avoids tracking errors that can occur on the tissue
167 boundary where cells have few neighbours.

168 Once the initial match (a 'seed') is found, the algorithm iteratively adds further cells to
169 the MCS. At each step of this iteration, a cell in the first network is picked that is adjacent to
170 the existing MCS and has a minimal number of potential matches. This number of potential
171 matches is determined based on how many cells in the second network have the same number of
172 neighbours as the considered cell while preserving connections to already tracked cells. Among
173 the choice of potential matches the algorithm identifies an optimal match based on the local
174 network structure of these cells' neighbours. A cell in the second network is identified as an
175 optimal match if the network structure of its neighbourhood is most similar to the cell in the
176 first match. This choice is made based on local MCSs between the neighbourhood-networks of
177 the cell in the first image and each potential match. Note that the optimal choice may exclude
178 the cell from the tracking entirely. In this case, most neighbours are included in the local MCS
179 when the considered cell is not tracked, indicating for example a cell removal event. In this
180 case the cell is not mapped and the algorithm proceeds by inspecting another cell in the first
181 match. Cells in the first frame for which no match in the second frame has been found may
182 be re-inspected at later stages of the algorithm as the size of the identified MCS increases.
183 Once no more adjacent cells can be added to the MCS through this iterative extension, the
184 iteration continues the search among untracked cells in the first network that are not adjacent
185 to the existing MCS. As soon as at least one cell has been added to the MCS in this way,
186 the algorithm again restricts its search to adjacent cells. The algorithm halts once no further
187 cell-to-cell matches can be found. During the construction of the MCS the algorithm ignores

188 any potential cell-to-cell matches where the corresponding cell centroids are more than a cutoff
189 distance d_{\max} apart within the field of view. Throughout the manuscript, we choose d_{\max} to be
190 ten average cell lengths.

191 Once the MCS is complete, any cells that have less than three isolated connections to other
192 cells in the MCS are removed from the tracking. Any clusters of ten or fewer cells are also
193 removed from the tracking result. Both of these steps help to minimise tracking errors (figure
194 2B-C).

195 **Cells are added to the tracking result by inspecting connections to previously**
196 **tracked neighbours** Through the identification of the MCS the algorithm tracks most of
197 the cells that do not rearrange between consecutive frames. Next, the algorithm tracks any re-
198 maining cells, and identifies cell rearrangements, cell removal, and cell division events. Similar
199 to the construction of the MCS, the tracking of remaining cells is iterative. At each iteration,
200 the algorithm identifies a cell-to-cell match that maximises the number of connections to al-
201 ready tracked cells, thus ‘growing’ the set of tracked cells from the intermediate tracking result
202 of the MCS. When adding cells to the tracking, the algorithm ensures that a cell cannot gain
203 more tracked neighbours between consecutive frames than the number of tracked neighbours
204 preserved between these frames. The algorithm also requires a cell to have at least two tracked
205 neighbours in order to be added to the tracking in this way.

206 Once all possible cells have been tracked, the algorithm resolves division events. Division
207 locations can be identified as regions in the second frame that contain more cells than the
208 corresponding region in the first frame. Since the algorithm will have found exactly one match
209 in the second network for each tracked cell in the first network and *vice versa*, there are thus
210 untracked cells in the second frame wherever a cell divides between two consecutive frames. The
211 algorithm attempts to resolve divisions events by identifying changes in cell-to-cell connectivity
212 that are characteristic to dividing cells (figure 4). For example, two cells adjacent to each
213 division must gain a neighbour (grey cells in figure 4A), and in many cases the mother and
214 daughter cells are easily identified as the cells that are shared neighbours of these cells adjacent
215 to the division event. However, one of the daughter cells may be four- or three-sided (figure
216 4B-C). In these cases, the algorithm is not able to determine the mother- and daughter cells
217 based on their network properties alone. Instead, the algorithm takes the geometric shape of

218 the cells into account. The mother and daughter cells are chosen by identifying which pair of
219 potential daughter cells has the closest position to their potential mother cell.

220 Cell deaths are identified as cells in the first frame that do not have a tracked match in the
221 second frame and that are not on the boundary of the region of tracked cells.

222 Code availability

223 The code used in this article is publicly available under the 3-clause BSD license as the
224 MCSTracker project (<https://github.com/kursawe/MCSTracker>). The project is implemented
225 in pure Python, employs unit testing [37] and is fully documented. Graphs in our code are rep-
226 resented using the Python package NetworkX [38].

227 Generation of *in silico* datasets

228 To test the algorithm, we generate *in silico* datasets that include examples of cell divisions,
229 removals and neighbour exchanges, as well as tissue movement. These datasets are generated
230 using Voronoi tessellations modified using Lloyd’s relaxation, which resemble cell packings in a
231 variety of epithelial tissues [33, 39].

232 To generate polygonal patterns of size $m \times n$, where m and n are natural numbers, $(m +$
233 $g) \times (n + g)$ Voronoi seeds are distributed uniformly at random in a 2D domain Ω of width $m + g$
234 and height $n + g$ (figure 5A). Here, g denotes the size of a boundary region that is introduced
235 to reduce the impact of the Voronoi boundary on the patterns. The domain Ω is surrounded
236 by two additional rows of evenly spaced seeds on each side. The inner row is a distance of 0.5
237 length units to Ω , and the seed-spacing is 1.0. The outer row has a distance of 1.5 to Ω , and
238 the seeds are shifted parallel to the first row by a distance of 0.5. The Voronoi tessellation of
239 all these seeds is then constructed.

240 In each Lloyd’s relaxation step, the polygons (or infinitely large areas) corresponding to the
241 regularly spaced seeds outside Ω are removed from the tessellation. Next, the centroid of each
242 remaining polygon is calculated and registered as a new seed. Further seeds are added that
243 again correspond to two rows of evenly spaced seeds outside Ω . A new Voronoi tessellation is
244 then constructed (figure 5B). This procedure is repeated for L relaxation steps, after which all
245 generated polygons are discarded except those whose centroids lie within a rectangular domain

246 of size $n \times m$ area units whose centroid coincides with that of Ω (figure 5B).

247 The polygonal tessellations have approximately $m \times n$ polygons of average area 1.0. During
248 the generation of the tessellations, evenly spaced seeds outside Ω are added to prevent the
249 occurrence of infinitely large polygons inside Ω . The boundary of size g is added in between
250 the generated tessellation and the evenly spaced seeds to reduce the effect of the evenly spaced
251 boundary seeds on the tessellation. Throughout this study, we use $g = 8$ and $n_L = 4$, resulting
252 in cell packings similar to those observed, for example, in the *Drosophila* wing imaginal disc [33].
253 We provide further details of how tissue rearrangements are implemented in the Results section.

254 **Experimental methods**

255 Live-imaging of cell proliferation was performed in stage-11 *Drosophila* embryos expressing a
256 tagged version of DE-Cadherin (DE-Cadherin::GFP) using a spinning disc confocal microscope,
257 as described in [40]. For the embryo setup, a modified version of the standard live-imaging
258 protocol was used [41].

259 **Data segmentation** Microscopy images were segmented using pixel classification in ilastik
260 [18]. The classifier was trained to recognise cell outlines and the segmentation of each frame
261 was manually corrected. A watershed algorithm was used to identify the precise shape of the
262 cell outlines. Each segmented frame was converted to a 16-bit grayscale image where pixels
263 belonging to different cells had different integer values. Polygonal tessellations for the tracking
264 algorithm were generated from the segmented image in two steps. First, all junctions between
265 three or more cells were identified as points where pixels of three or more different cells met;
266 second, vertices were assigned to cells. Then, edges shorter than two pixels (0.5 μm) were
267 removed and replaced by a single vertex at the midpoint of the edge. Finally, polygons at
268 the boundary of the tissue were removed from the simulation. This removal was necessary
269 since cell shapes at the tissue boundary are poorly approximated by polygons due to missing
270 vertices. Note that our algorithm can interpret segmentations saved using either ilastik [18] or
271 SeedWaterSegmenter [10].

272 3 Results

273 ***In silico* testing of the algorithm.** To assess the performance of the algorithm, we begin
274 by applying it to *in silico* datasets that include cell neighbour exchanges, tissue movement, cell
275 removal and cell division, respectively. In each case, we compare the outcome of the tracking
276 algorithm to the ground truth.

277 We begin by assessing the ability of the algorithm to resolve permutations in otherwise
278 identical tissues (figure 6A). In this test, a random tessellation of size nine by nine cells is
279 created as described in the Methods section, and integer identifiers c_i are assigned to each cell.
280 Next, an identical copy of the tissue is created in which the integer identifiers are randomly
281 shuffled. A ground truth mapping from the first to the second integer identifiers is generated.
282 Next, the algorithm is applied. Upon conducting 100 such tests, we find that all identified
283 cell-to-cell mappings are matched correctly, as compared to the ground truth. In rare examples,
284 isolated cells at the boundary of the tissue are not tracked. In these examples, either a
285 single cell has only one adjacent cell in the tissue, or two cells of identical polygon number
286 are adjacent and share exactly one neighbour. Neither the MCS detection algorithm, nor the
287 post-processing algorithm are able to resolve such mappings, which involve fewer than four cells
288 in each dataset (fewer than five percent of the tissue).

289 We design four further tests of tissue rearrangements (figure 6B-E). The first test comprises
290 tissue movements between images (figure 6B). In this test, a tissue of size fifteen by eight cells
291 is generated as described in the Methods section. Two smaller tissues of width seven units are
292 cut out of this tissue, which each cover the full height of the tissue, and which are horizontally
293 translated relative to each other by a distance of two cell lengths. The position of each three-cell
294 junction in both tissues is shifted such that the x -coordinate of the left-most junction in each
295 tissue is 0.

296 The second test (figure 6C) generates cell neighbour exchanges, also called T1 transitions
297 [42, 43]. In our implementation of T1 transitions, an edge shared by two cells is replaced by a
298 new perpendicular edge (of length $l_{T1} = 0.2$ units) such that the local cell connectivity changes
299 (figure 2B). We create two identical copies of a tissue of size nine by nine cells. In the second
300 copy, a T1 transition is performed on an edge in the centre of the tissue.

301 The third test involves cell removal (figure 6D). In this test, we first generate two identical

302 copies of a tissue of size nine by nine cells. In the second copy, we replace the central cell by a
303 vertex shared by its neighbouring cells, a rearrangement similar to so-called T2 transitions [42].
304 The final test involves cell divisions (figure 6E). Here, we once again create two identical copies
305 of size nine by nine cells. In the second copy, a cell in the centre of the tissue is bisected by
306 introducing a straight line in a random direction through the centroid of that cell.

307 For all tests generated in this way, integer cell identifiers in the second tissue are randomly
308 shuffled, and a ground truth is generated. We run 100 realisations of each test case, and compare
309 the tracking outcome to the ground truth. In all cases we find that cells are tracked correctly,
310 with at most three unmatched cells at the boundary of the sheet.

311 In figure 6, all cells identified after the cleaning step, in which weakly connected cells are re-
312 moved from the MCS, are coloured green, whereas cells that are identified by the post-processing
313 algorithm are coloured red. Note that the exact number of cells that are identified by the post-
314 processing algorithm varies between individual realisations of the tests. In many cases, the cells
315 identified by the post-processing algorithm include cells that are adjacent to those undergoing
316 division, removal or neighbour exchange.

317 We next analyse the extent to which the success of our tracking algorithm depends on the
318 number of Lloyd's relaxation steps, n_L , used to generate the *in silico* datasets. To investigate
319 this we iteratively increase n_L , thus generating tissues with increasingly homogeneous graph
320 structures, and repeat all tests. We find that the algorithm successfully passes all tests for all
321 values of n_L from 4 up to 14.

322 **Algorithm performance for large numbers of cell neighbour exchanges** To assess the
323 performance of the algorithm when applied to tissues exhibiting large numbers of cell neighbour
324 exchanges, we next apply the algorithm to *in silico* datasets with increasing numbers of cell
325 neighbour exchanges between frames (figure 7). The number of correctly tracked cells decreases
326 as the number of cell neighbour exchanges increases. However, the number of incorrectly tracked
327 cells remains below 20% throughout the analysed range of neighbour exchanges, and decreases
328 to zero as the number of edge swaps exceeds 10%.

329 The number of untracked cells increases rapidly as the percentage of cell-cell interfaces
330 that are swapped between successive images increases from five to ten percent. Note that the
331 percentage of cells involved in these neighbour exchanges is larger than the percentage of cell-

332 cell interfaces that are swapped, since an individual T1 transition changes the cell neighbour
333 relations of four cells, and each cell shares multiple inner edges. For example, rearranging five
334 percent of the inner edges of the tissue affects roughly 40% of the cells in the tissue, while
335 rearranging ten percent of the tissue edges affects up to 70% of the cells. The number of
336 (correctly or incorrectly) tracked cells drops to zero if the tissue rearranges so much that the
337 neighbourhood of each cell changes; in this case a first match cannot be found to initialise the
338 MCS construction algorithm.

339 **Application of the algorithm to *in vivo* data** Figure 8 shows the first three of 21 seg-
340 mented image frames of the lateral epidermis of a stage-11 *Drosophila* embryo to which the
341 algorithm was applied. During stage 11, gross morphogenetic movements do not occur but the
342 tissue is very active with a large number of proliferations occurring within a short duration,
343 making this a much more challenging tissue on which to perform cell segmentation and tracking
344 than the wing imaginal disc, where many previous efforts have been made [4, 13, 31, 44]. Cell
345 delamination is also more common than in the wing imaginal disc during normal development.
346 This stage of development thus offers a true test of the present algorithm.

347 The images were taken five minutes apart over a time span of 100 minutes. These first three
348 images comprise 271, 263 and 263 cells, respectively. Our algorithm tracks 247 cells between
349 the first and second images, 245 cells between the second and third images and 234 cells across
350 all three images. The centroids of cells of previous images are superimposed on the tracking
351 results in figure 8, illustrating that the algorithm successfully tracks cells in situations where
352 it is difficult to match cells between images based on the centroid positions alone. Cells that
353 include only their corresponding centroid from the previous image are coloured in green, while
354 cells that do not include their corresponding centroid from the previous image, and cells that
355 include multiple centroids from the previous image, are coloured in purple. In the first frame
356 we highlight ‘higher-order’ junctions (shared by four or more cells) by yellow asterisks. Such
357 junctions occur frequently throughout the dataset.

358 On average, cell centroids move 0.75 cell lengths between the first and second images, with a
359 maximal displacement of 1.17 cell lengths. Between the first and second images 36 cells undergo
360 a net gain in edges, whereas 20 cells have a net loss of edges. In total, four cell deaths and no
361 cell divisions are observed across the three data images. Inspection of all individual cell tracks

362 reveals that none of the cells are tracked incorrectly.

363 The data in figure 8 are the first three out of 21 frames. In figure 9 we show the results
364 of the analysis of the full dataset, including all 21 frames. During the period of measurement
365 the total number of cells increases from 280 to 330 cells, whereas the total number of tracked
366 cells increases from 270 to roughly 310 cells. As the number of cells in the tissue rises, the
367 total number of cell rearrangements increases, whereas the average cell area decreases. Here,
368 the number of cell rearrangements is measured by counting how many cells change their cell-
369 neighbour number between consecutive frames. For all frames, the number of tracked cells is
370 lower than the number of cells in the tissue. A visual inspection of the tracked data reveals that
371 the difference between the total number of cells and the number of tracked cells is largely due
372 to cells entering or leaving the field of view. The percentage of cells that our algorithm tracks
373 is lowest (84%) when the rates of cell division and cell rearrangement are highest, which occurs
374 at 70 minutes. Here, the number of tracked cells decreases since the algorithm is not yet able
375 to resolve division events immediately adjacent to rearrangements as well as multiple adjacent
376 divisions.

377 Since cell rearrangements are one of the most difficult aspects of cell tracking, and our *in*
378 *vivo* data exhibit a high frequency of such events, it is natural to ask what percentage of cells
379 are correctly tracked. To estimate this percentage, we compare the results in figure 9, where up
380 to 30% of cells are involved in neighbour exchange between frames in a population of up to 340
381 cells, with the results shown in figure 7, where in the case of 400 cells and 4% of edges undergoing
382 T1 transitions between frames (corresponding to 30% of cells involved in neighbour exchanges)
383 we find the percentage of correctly tracked cells to be 85%. This provides a lower bound for
384 the success rate of the algorithm on the *in vivo* frames. When up to 3% of edges undergo T1
385 transitions (corresponding to 25% of cells in the tissue involved in neighbour exchanges), the
386 success rate of the algorithm is 98%.

387 The tracking of epithelial *in vivo* data enables quantitative assessment of dynamic changes
388 in cellular morphology. The tracking results in figure 9 reveals that the analysed section of
389 the epidermis undergoes 60 cell rearrangements per five minutes initially and around 100 cell
390 rearrangements per five minutes at the end of the observed time interval. The average ratio
391 of the maximal area and the minimal area observed for individual cells during the period of

392 measurement is 4.2, indicating that on average cells increase their apical area by a factor of four
393 during mitotic rounding. A total of 18 cell deaths are tracked in the dataset. A striking feature
394 is the level of T1 transitions occurring during this stage of development, even in the absence of
395 gross morphogenetic movements found in earlier or later stages.

396 **Calculation times** To analyse the scaling of the calculation times with tissue size we repeat
397 the permutation test with tissues of square dimension of varying size on a desktop computer
398 with an Intel i5-6500T CPU (2.5GHz) and 8GB RAM. We find that the calculation times scale
399 subquadratically with cell number (figure 10).

400 The calculation times for the experimental images analysed in figure 8 vary more widely
401 than for the *in silico* datasets. For the tracking between the first and second frames in figure
402 8, the algorithm required 43 seconds to run, whereas between the second and the third frames
403 the algorithm required 9 seconds. This is due to differences in the time required to find the first
404 correct mapping; in the first example 154 cells were searched before the first correct mapping
405 was found, whereas in the second example only 12 cells were searched. This means that the
406 number of cells considered when finding the initial mappings depends on the graph structure of
407 the analysed frames and impacts on the calculation time of the algorithm. In total, analysing
408 all 21 frames of the *in vivo* data presented in figures 8 and 9 requires 19 minutes of calculation
409 time.

410 4 Discussion

411 Cell tracking in epithelial sheets has the potential to generate a vast amount of quantitative data
412 to inform our understanding of the contributions of different cellular processes to tissue morpho-
413 genesis. However, cell tracking is notoriously difficult, especially for the complex morphogenetic
414 processes that occur as embryogenesis proceeds. Here, we present an algorithm based on MCS
415 detection for the tracking of cells in segmented images of epithelial sheets. Our algorithm suc-
416 cessfully tracks cells in *in vivo* images of the *Drosophila* embryonic epidermis, a challenging
417 dataset compared to other tissues, as well as in randomly generated *in silico* datasets, without
418 the need for the adjustment of tissue specific parameters such as weights for individual terms
419 in a global minimisation scheme [14]. The use of *in silico* data to test our algorithm allows us

420 to analyse the performance of our algorithm for a large range of experimentally observed cell
421 rearrangements and tessellations.

422 The tracking of cells in *in vivo* datasets such as presented in figures 8 and 9 provides quan-
423 titative insight into tissue homeostasis. Using our algorithm we measure example quantities
424 that would not be accessible without a robust cell tracking method. The amount of cell re-
425 arrangement, the extent of mitotic rounding, and the occurrence of cell death in the observed
426 frames each can be used to learn about tissue homeostasis in developing epithelia. Within the
427 analysed dataset, we find a significant number of T1 transitions despite the absence of gross
428 morphogenetic movements. This may be driven by the large number of proliferation events that
429 occur. Further, using *in vivo* imaging together with our tracking algorithm allows the obser-
430 vation of cell death or cell delamination without the need for fluorescent markers of apoptosis.
431 Future applications of the algorithm to such processes may, for example, provide novel insight
432 to tissue size control in the *Drosophila* embryonic epidermis [9,45] and can also be adapted to
433 study the dynamics of epithelial wound closure. In this and other systems cell tracking may
434 enable the observation of cell death due to delamination as opposed to apoptosis [46]. Access
435 to quantification of cell rearrangement and area changes has recently provided insight to wing
436 morphogenesis in *Drosophila* [43].

437 Our algorithm is able to track cells that undergo significant movement and neighbour ex-
438 changes between frames. For example, we can correctly track cells in tissues where more than
439 40% of the cells rearrange between successive movie frames (figure 7). In addition, even com-
440 parably large gaps in the initial MCS can be filled in during the post-processing step (figures
441 2 and 8). For example, in the first tracking step in figure 1, only 182 of the 246 tracked cells
442 were identified by the MCS algorithm, and it was possible to track the 64 remaining cells during
443 the post-processing step. For comparison, Heller et al [13] report 15 cell rearrangements per
444 1000 cells per hour at an imaging interval of six minutes for their time-lapse microscopy data of
445 *Drosophila* wing imaginal discs. In addition, the experimental data shown in figures 2, 8, and
446 9 include junctions shared by four or more cells (yellow asterisks in figure 9) while our *in silico*
447 data include multiple instances of such junctions (figure 6D). Therefore, higher-order junctions,
448 such as multicellular rosettes [47,48], do not pose a challenge to our algorithm.

449 Our algorithm is able to correctly track cells in all considered test cases. However, on rare

450 occasions a few cells at the tissue boundary cannot be tracked. It may be possible to adapt the
451 algorithm to track these cells, if this is considered necessary for the application at hand. In the
452 current version of the algorithm, two connections to already tracked cells that are preserved
453 between two time frames are a condition to add a cell-to-cell mapping in the post-processing
454 algorithm. Further analysis of cases where this condition is not fulfilled may reveal ways to
455 relax it.

456 When generating *in silico* data to test the algorithm, we used Voronoi tessellations in com-
457 bination with Lloyd's relaxation to generate data that resembles tissues in the *Drosophila* wing
458 imaginal disc [33]. We expect the algorithm to perform less well on tissues whose network
459 structure is nearly homogeneous. For example, in an epithelial sheet where cells are arranged
460 in a hexagonal fashion, such as the early *Drosophila* embryonic epidermis [49] or the late pu-
461 pal *Drosophila* wing [50], the local adjacency network of each cell is identical, and hence a
462 network-based tracking algorithm may not be able to distinguish cells. When generating *in*
463 *silico* tissues, we use four Lloyd's relaxation steps after Voronoi tessellation. With each Lloyd's
464 relaxation step, the homogeneity of the tissue increases. We were able to successfully repeat
465 all *in silico* tests on virtual tissues that were generated using up to 14 Lloyd's relaxation steps.
466 Hence, we expect the algorithm to be suitable for tissues that can be well described with 14
467 or fewer Lloyd's relaxation steps, such as the chick neural tube embryonic epithelium, or the
468 *Drosophila* eye disc [33].

469 The algorithm relies on being able to generate polygonal tessellations from segmented video
470 microscopy data. In particular, all *in silico* tests we conducted consider tissues where each cell
471 has at least three neighbours. Conceptually, it would be possible to apply the algorithm to
472 tissues in which individual cells may have only two neighbours, although such examples have
473 not been included in the present analysis.

474 In microscopy videos including division events we expect the algorithm to perform well in
475 tissues in which no adjacent divisions occur between successive movie frames, and in which
476 cells adjacent to the dividing cell do not undergo rearrangements before the next frame is
477 captured. Our algorithm is designed to identify mother and daughter cells of a division event
478 by establishing the bordering cells that gain an edge during the division event. In the case of two
479 adjacent divisions, and if cells adjacent to a division event gain edges due to cell rearrangements,

480 the dividing cell cannot be correctly identified. An example of a typical tracking error for two
481 adjacent divisions is shown in figure 11. In cases where the division resolution step fails, our
482 Python implementation returns all tracked cells of the post-processing step, and gives a warning
483 that the division has not been resolved. In these cases, manual correction methods could be
484 used for incorrectly tracked cells in the vicinity of division events.

485 The parameters of the algorithm are chosen to maximise its robustness and avoid the neces-
486 sity to adjust the parameters to individual applications. For example, the cutoff length d_{\max} ,
487 that determines the distance below which two cells in consecutive movie frames are considered
488 mappable to each other, was chosen to be 10 times the average cell length in the tissue, which
489 is significantly larger than the movement that is to be expected between consecutive frames of
490 a live-imaging microscopy video. However, parameter adjustments may be possible for individ-
491 ual applications in order to decrease the algorithm calculation times. For example, the size of
492 the extended neighbourhood considered in the initial step or the iterative extension could be
493 reduced to include only nearest neighbours instead of nearest neighbours and second nearest
494 neighbours in case the tissue is sufficiently heterogeneous. Similarly, one might decrease the
495 d_{\max} for possible cell pairings if the cell positions are not expected to vary significantly between
496 time frames.

497 Adjustments may be possible to extend the applicability of the algorithm to a wider range of
498 tissues. For example, instead of automatic detection of the initial seeds for the MCS detection
499 algorithm, a small set of seeds could be manually supplied to guide the tracking. This should
500 improve the performance of the algorithm on homogeneous tissues. In such cases, irregular
501 boundaries may also help to aid the initial seeding. During the construction of the MCS, non-
502 adjacent cells are considered for addition to the MCS whenever the extension of the MCS by
503 adjacent cells is not possible. An alternative option to extend an intermediate MCS may be to
504 repeat the initial seeding algorithm.

505 In the present work, we have sought to keep geometrical input to the algorithm to a mini-
506 mum. Cases where geometric data are taken into account comprise division events where one of
507 the daughter cells is four- or three- sided, since in these cases we are not able to make a decision
508 on which cell is the second daughter cell based on network adjacency alone. If future applica-
509 tions reveal cases where the algorithm performs poorly due to a large number of cell neighbour

510 exchanges or high degree of tissue homogeneity, then it may be possible to construct algorithms
511 that combine information on the network topology with data on cell shapes, cell positions and
512 cell movements to improve performance. For example, information on network topology could
513 be integrated into previous algorithms that minimise differences between geometric properties
514 of cells, such as cell size and location [24], with information about network connectivity.

515 In cell tracking applications, the scaling of the algorithm with tissue size is crucial. Potential
516 applications range from systems of 30 cells (*Drosophila* embryonic epidermal P compartments
517 [9]), to 10,000 cells (*Drosophila* imaginal wing disc [31]; the wing pouch has about 3,000 cells
518 [40]). Calculation times in the presented algorithm scale subquadratically with cell number,
519 making it suitable for applications of varying sizes. For example, extrapolating the data in
520 figure 10, a tissue of 10,000 cells could be tracked across two frames within 10 minutes. The
521 scaling of the algorithm is polynomial despite the fact that it is based on MCS detection, which
522 is known to scale exponentially in the general case. MCS detection has a wide range of research
523 applications, including protein interaction networks [51, 52] and finding the binding sites of
524 chemical structures [53]. Our approach of reducing the MCS search to a localised search may
525 have applications in other areas where the networks are inherently planar.

526 Our algorithm is designed to track cells in segmented microscopy videos of epithelial sheets
527 in two dimensions. However, it may be possible to apply the algorithm to datasets of epithelial
528 sheets that are embedded in a three-dimensional environment, such as the *Drosophila* imaginal
529 wing disc [4], or the *Drosophila* embryonic epidermis [6,9], including tissues that can be mapped
530 onto a cylinder or ellipsoid, such as the mouse visceral endoderm [48].

531 A large number of cell tracking algorithms have been developed for various applications [10–
532 27]. Further efforts are required to compare these algorithms with our own, and to identify the
533 algorithm best suited for an individual dataset. In the cell tracking challenge [54] the authors
534 provide microscopy videos from a variety of *in vitro* cell cultures, including, for example, mouse
535 embryonic stem cells and human squamous lung carcinoma cells, together with ground-truth
536 segmentation and tracking data as benchmarks for cell tracking and segmentation algorithms.
537 However, many of the published algorithms above have not yet been applied to the challenge,
538 and benchmark datasets for epithelial sheets are not currently available. The fully segmented
539 dataset published within the MCSTracker project can provide a benchmark for future epithelial

540 cell tracking applications. In [55] *in silico* datasets are used as benchmarking datasets for
541 particle tracking algorithms.

542 The proposed algorithm provides a two-dimensional tracking solution specialised for epithe-
543 lial sheets that attempts to maximise the information that can be gained from the packing that
544 is typical to such tissues. It may, however, be possible to extend this algorithm to applications of
545 cell tracking where cells are not physically connected by constructing adjacency networks from
546 Voronoi tessellations that use the cell locations as seeds. We hope that, as segmentation tools
547 are developed further, the combination of our algorithm with these tools will lead to further
548 insights into cellular behaviour in epithelial tissues.

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554 References

- 555 [1] Stephens, D. J. and Allan, V. J. Light microscopy techniques for live cell imaging. *Science*,
556 300(5616):82–86, 2003. 10.1126/science.1082160.
- 557 [2] Pantazis, P. and Supatto, W. Advances in whole-embryo imaging: a quantitative transition
558 is underway. *Nat. Rev. Mol. Cell. Biol.*, 15(5):327–339, 2014. 10.1038/nrm3786.
- 559 [3] Truong, T. V. and Supatto, W. Toward high-content/high-throughput imaging and analysis
560 of embryonic morphogenesis. *Genesis*, 49(7):555–569, 2011. 10.1002/dvg.20760.
- 561 [4] Mao, Y., Tournier, A. L., Bates, P. A., Gale, J. E., Tapon, N., and Thompson, B. J. Planar
562 polarization of the atypical myosin Dachs orients cell divisions in *Drosophila*. *Genes Dev.*,
563 25(2):131–136, 2011. 10.1101/gad.610511.

- 564 [5] Gibson, M. C., Patel, A. B., Nagpal, R., and Perrimon, N. The emergence of geometric
565 order in proliferating metazoan epithelia. *Nature*, 442(7106):1038–1041, 2006. 10.1038/
566 nature05014.
- 567 [6] Rauzi, M., Verant, P., Lecuit, T., and Lenne, P.-F. Nature and anisotropy of cortical
568 forces orienting *Drosophila* tissue morphogenesis. *Nat. Cell Biol.*, 10(12):1401–1410, 2008.
569 10.1038/ncb1798.
- 570 [7] Collinet, C., Rauzi, M., Lenne, P.-F., and Lecuit, T. Local and tissue-scale forces drive
571 oriented junction growth during tissue extension. *Nat. Cell Biol.*, 17(10):1247–1258, 2015.
572 10.1038/ncb3226.
- 573 [8] Ritsma, L., Ellenbroek, S. I. J., Zomer, A., Snippert, H. J., de Sauvage, F. J., Simons, B. D.,
574 Clevers, H., and van Rheenen, J. Intestinal crypt homeostasis revealed at single-stem-cell
575 level by *in vivo* live imaging. *Nature*, 507(7492):362–365, 2014. 10.1038/nature12972.
- 576 [9] Parker, J. Control of compartment size by an EGF ligand from neighboring cells. *Curr.*
577 *Biol.*, 16(20):2058–2065, 2006. 10.1016/j.cub.2006.08.092.
- 578 [10] Mashburn, D. N., Lynch, H. E., Ma, X., and Hutson, M. S. Enabling user-guided seg-
579 mentation and tracking of surface-labeled cells in time-lapse image sets of living tissues.
580 *Cytometry A*, 81A(5):409–418, 2012. 10.1002/cyto.a.22034.
- 581 [11] Cilla, R., Mechery, V., Hernandez de Madrid, B., Del Signore, S., Dotu, I., and Hatini, V.
582 Segmentation and tracking of adherens junctions in 3D for the analysis of epithelial tissue
583 morphogenesis. *PLoS Comput. Biol.*, 11(4):e1004124, 2015. 10.1371/journal.pcbi.1004124.
- 584 [12] Schiegg, M., Hanslovsky, P., Kausler, B., Hufnagel, L., and Hamprecht, F. Conservation
585 tracking. *IEEE Int. Comp. Vis.*, 2928–2935, 2013. 10.1109/ICCV.2013.364.
- 586 [13] Heller, D., Hoppe, A., Restrepo, S., Gatti, L., Tournier, A., Tapon, N., Basler, K., and
587 Mao, Y. EpiTools: An open-source image analysis toolkit for quantifying epithelial growth
588 dynamics. *Dev. Cell*, 36(1):103–116, 2016. 10.1016/j.devcel.2015.12.012.
- 589 [14] Padfield, D., Rittscher, J., and Roysam, B. Coupled minimum-cost flow cell track-
590 ing for high-throughput quantitative analysis. *Med. Image Anal.*, 15(4):650–668, 2011.
591 10.1016/j.media.2010.07.006.

- 592 [15] Youssef, S., Gude, S., and Radler, J. O. Automated tracking in live-cell time-lapse movies.
593 *Integr. Biol.*, 3:1095–1101, 2011. 10.1039/C1IB00035G.
- 594 [16] Wait, E., Winter, M., Bjornsson, C., Kokovay, E., Wang, Y., Goderie, S., Temple, S., and
595 Cohen, A. Visualization and correction of automated segmentation, tracking and lineaging
596 from 5-D stem cell image sequences. *BMC Bioinform.*, 15(1):328, 2014. 10.1186/1471-2105-
597 15-328.
- 598 [17] Winter, M., Wait, E., Roysam, B., Goderie, S. K., Ali, R. A. N., Kokovay, E., Temple, S.,
599 and Cohen, A. R. Vertebrate neural stem cell segmentation, tracking and lineaging with
600 validation and editing. *Nat. Protocols*, 6(12):1942–1952, 2011. 10.1038/nprot.2011.422.
- 601 [18] Sommer, C., Straehle, C., Köthe, U., and Hamprecht, F. A. Ilastik: Interactive learning
602 and segmentation toolkit. In *IEEE International Symposium on Biomedical Imaging: From
603 Nano to Macro*, 230–233. 2011. 10.1109/ISBI.2011.5872394.
- 604 [19] Liu, K., Lienkamp, S. S., Shindo, A., Wallingford, J. B., Walz, G., and Ronneberger,
605 O. Optical flow guided cell segmentation and tracking in developing tissue. In
606 *IEEE 11th International Symposium on Biomedical Imaging (ISBI)*, 298–301. 2014.
607 10.1109/ISBI.2014.6867868.
- 608 [20] Bellaiche, Y., Bosveld, F., Graner, F., Mikula, K., Remesikova, M., and Smisek, M. New
609 robust algorithm for tracking cells in videos of *Drosophila* morphogenesis based on finding
610 an ideal path in segmented spatio-temporal cellular structures. In *IEEE Annu. Int. Conf.
611 Eng. Med. Biol. Soc.*, 6609–6612. 2011. 10.1109/IEMBS.2011.6091630.
- 612 [21] Aly, A. A., Deris, S. B., and Zaki, N. Intelligent algorithms for cell tracking and image
613 segmentation. *Int. J. Comput. Sci. Inf. Technol.*, 6(5):21–37, 2014.
- 614 [22] Wang, Q., Niemi, J., Tan, C.-M., You, L., and West, M. Image segmentation and dynamic
615 lineage analysis in single-cell fluorescence microscopy. *Cytometry A*, 77A(1):101–110, 2010.
616 10.1002/cyto.a.20812.
- 617 [23] Raffel, M., Willert, C. E., Wereley, S., and Kompenhans, J. *Particle Image Velocimetry:
618 A Practical Guide*. Springer-Verlag Berlin Heidelberg, 2007.

- 619 [24] Puliafito, A., Hufnagel, L., Neveu, P., Streichan, S., Sigal, A., Fygenson, D. K., and
620 Shraiman, B. I. Collective and single cell behavior in epithelial contact inhibition. Proc.
621 Natl. Acad. Sci. U.S.A., 109(3):739–744, 2012. 10.1073/pnas.1007809109.
- 622 [25] Al-Kofahi, Y., Lassoued, W., Lee, W., and Roysam, B. Improved automatic detection
623 and segmentation of cell nuclei in histopathology images. IEEE Trans. Biomed. Eng.,
624 57(4):841–852, 2010. 10.1109/TBME.2009.2035102.
- 625 [26] Amat, F., Lemon, W., Mossing, D. P., McDole, K., Wan, Y., Branson, K., Myers, E. W.,
626 and Keller, P. J. Fast, accurate reconstruction of cell lineages from large-scale fluorescence
627 microscopy data. Nat. Meth., 11(9):951–958, 2014. 10.1038/nmeth.3036.
- 628 [27] Aigouy, B., Farhadifar, R., Staple, D. B., Sagner, A., Röper, J.-C., Jülicher, F., and Eaton,
629 S. Cell flow reorients the axis of planar polarity in the wing epithelium of *Drosophila*. Cell,
630 142(5):773–786, 2010. 10.1016/j.cell.2010.07.042.
- 631 [28] Hoebe, R. A., Van Oven, C. H., Gadella, T. W. J., Dhonukshe, P. B., Van Noorden, C.
632 J. F., and Manders, E. M. M. Controlled light-exposure microscopy reduces photobleaching
633 and phototoxicity in fluorescence live-cell imaging. Nat. Biotech., 25(2):249–253, 2007.
634 10.1038/nbt1278.
- 635 [29] Wood, W. and Jacinto, A. Cell Migration: Developmental Methods and Protocols, chapter
636 Imaging Cell Movement During Dorsal Closure in *Drosophila* Embryos, 203–210. Humana
637 Press, Totowa, NJ, 2005. 10.1385/1-59259-860-9:203.
- 638 [30] Mavrakakis, M., Rikhy, R., Lilly, M., and Lippincott-Schwartz, J. Fluorescence Imaging
639 Techniques for Studying *Drosophila* Embryo Development. John Wiley & Sons, Inc., 2001.
640 10.1002/0471143030.cb0418s39.
- 641 [31] Farhadifar, R., Röper, J.-C., Aigouy, B., Eaton, S., and Jülicher, F. The influence of
642 cell mechanics, cell-cell interactions, and proliferation on epithelial packing. Curr. Biol.,
643 17(24):2095–2104, 2007. 10.1016/j.cub.2007.11.049.
- 644 [32] Escudero, L. M., da F. Costa, L., Kicheva, A., Briscoe, J., Freeman, M., and Babu, M. M.
645 Epithelial organisation revealed by a network of cellular contacts. Nat. Commun., 2:526,
646 2011. 10.1038/ncomms1536.

- 647 [33] Sánchez-Gutiérrez, D., Tozluoglu, M., Barry, J. D., Pascual, A., Mao, Y., and Escudero,
648 L. M. Fundamental physical cellular constraints drive self-organization of tissues. *EMBO*
649 *J.*, 2015. 10.15252/embj.201592374.
- 650 [34] Sáez, A., Acha, B., Montero-Sánchez, A., Rivas, E., Escudero, L. M., and Serrano, C.
651 Neuromuscular disease classification system. *J. Biomed. Opt.*, 18(6):066017–066017, 2013.
652 10.1117/1.JBO.18.6.066017.
- 653 [35] Ullmann, J. R. An algorithm for subgraph isomorphism. *J. ACM*, 23(1):31–42, 1976.
654 10.1145/321921.321925.
- 655 [36] Krissinel, E. B. and Henrick, K. Common subgraph isomorphism detection by backtracking
656 search. *Software Pract. Exper.*, 34(6):591–607, 2004. 10.1002/spe.588.
- 657 [37] Osborne, J. M., Bernabeu, M. O., Bruna, M., Calderhead, B., Cooper, J., Dalchau, N.,
658 Dunn, S.-J., Fletcher, A. G., Freeman, R., Groen, D., et al. Ten simple rules for effec-
659 tive computational research. *PLoS Comput. Biol.*, 10(3):e1003506, 2014. 10.1371/jour-
660 nal.pcbi.1003506.
- 661 [38] Hagberg, A. A., Schult, D. A., and Swart, P. J. Exploring network structure, dynamics,
662 and function using NetworkX. In *Proceedings of the 7th Python in Science Conference*
663 *(SciPy2008)*, 11–15. Pasadena, CA USA, 2008.
- 664 [39] Honda, H. Description of cellular patterns by Dirichlet domains: The two-dimensional
665 case. *J. Theor. Biol.*, 72(3):523–543, 1978. 10.1016/0022-5193(78)90315-6.
- 666 [40] Narciso, C., Wu, Q., Brodskiy, P. A., Garston, G., Baker, R. E., Fletcher, A. G., and
667 Zartman, J. J. Patterning of wound-induced intercellular Ca²⁺ flashes in a developing
668 epithelium. *Phys. Biol.*, 12(5):056005, 2015. 10.1088/1478-3975/12/5/056005.
- 669 [41] Parton, R. M., Vallés, A. M., Dobbie, I. M., and Davis, I. Collection and mounting of
670 *Drosophila* embryos for imaging. *Cold Spring Harbor Protocols*, 2010(4):pdb.prot5403,
671 2010. 10.1101/pdb.prot5403.
- 672 [42] Nagai, T., Kawasaki, K., and Nakamura, K. Vertex dynamics of two-dimensional cellular
673 patterns. *J. Phys. Soc. Jpn.*, 57(7):2221–2224, 1988. 10.1143/JPSJ.57.2221.

- 674 [43] Etournay, R., Popović, M., Merkel, M., Nandi, A., Blasse, C., Aigouy, B., Brandl, H.,
675 Myers, G., Salbreux, G., Jülicher, F., et al. Interplay of cell dynamics and epithe-
676 lial tension during morphogenesis of the *Drosophila* pupal wing. *eLife*, 4:e07090, 2015.
677 10.7554/eLife.07090.
- 678 [44] Aegerter-Wilmsen, T., Smith, A. C., Christen, A. J., Aegerter, C. M., Hafen, E., and Basler,
679 K. Exploring the effects of mechanical feedback on epithelial topology. *Development*,
680 137(3):499–506, 2010. 10.1242/dev.041731.
- 681 [45] Kursawe, J., Brodskiy, P. A., Zartman, J. J., Baker, R. E., and Fletcher, A. G. Capabilities
682 and limitations of tissue size control through passive mechanical forces. *PLoS Comput.*
683 *Biol.*, 11(12):1–26, 2016. 10.1371/journal.pcbi.1004679.
- 684 [46] Marinari, E., Mehonic, A., Curran, S., Gale, J., Duke, T., and Baum, B. Live-cell delamina-
685 tion counterbalances epithelial growth to limit tissue overcrowding. *Nature*, 484(7395):542–
686 545, 2012. 10.1038/nature10984.
- 687 [47] Blankenship, J. T., Backovic, S. T., Sanny, J. S. P., Weitz, O., and Zallen, J. A. Multicellu-
688 lar Rosette Formation Links Planar Cell Polarity to Tissue Morphogenesis. *Developmental*
689 *Cell*, 11(4):459–470, 2006. 10.1016/j.devcel.2006.09.007.
- 690 [48] Trichas, G., Smith, A. M., White, N., Wilkins, V., Watanabe, T., Moore, A., Joyce,
691 B., Sugnaseelan, J., Rodriguez, T. A., Kay, D., et al. Multi-cellular rosettes in the mouse
692 visceral endoderm facilitate the ordered migration of anterior visceral endoderm cells. *PLoS*
693 *Biol.*, 10(2):e1001256, 2012. 10.1371/journal.pbio.1001256.
- 694 [49] Warn, R. and Magrath, R. F-actin distribution during the cellularization of the
695 *drosophila* embryo visualized with FL-phalloidin. *Exp. Cell Res.*, 143(1):103 – 114, 1983.
696 [http://dx.doi.org/10.1016/0014-4827\(83\)90113-1](http://dx.doi.org/10.1016/0014-4827(83)90113-1).
- 697 [50] Classen, A.-K., Anderson, K. I., Marois, E., and Eaton, S. Hexagonal packing of *Drosophila*
698 wing epithelial cells by the planar cell polarity pathway. *Dev. Cell*, 9(6):805–817, 2005.
699 10.1016/j.devcel.2005.10.016.

- 700 [51] Ciriello, G., Mina, M., Guzzi, P. H., Cannataro, M., and Guerra, C. Alignnemo: A local
701 network alignment method to integrate homology and topology. *PLoS ONE*, 7(6):e38107,
702 2012. 10.1371/journal.pone.0038107.
- 703 [52] Aladağ, A. E. and Erten, C. Spinal: scalable protein interaction network alignment. *Bioin-*
704 *formatics*, 29(7):917–924, 2013. 10.1093/bioinformatics/btt071.
- 705 [53] Raymond, J. W. and Willett, P. Maximum common subgraph isomorphism algorithms for
706 the matching of chemical structures. *J. Comput.-Aided Mol. Des.*, 16(7):521–533, 2002.
707 10.1023/A:1021271615909.
- 708 [54] Maška, M., Ulman, V., Svoboda, D., Matula, P., Matula, P., Ederra, C., Urbiola, A.,
709 España, T., Venkatesan, S., Balak, D. M., et al. A benchmark for comparison of cell track-
710 ing algorithms. *Bioinformatics*, 30(11):1609–1617, 2014. 10.1093/bioinformatics/btu080.
- 711 [55] Chenouard, N., Smal, I., de Chaumont, F., Maska, M., Sbalzarini, I. F., Gong, Y., Car-
712 dinale, J., Carthel, C., Coraluppi, S., Winter, M., et al. Objective comparison of particle
713 tracking methods. *Nat. Meth.*, 11(3):281–289, 2014. 10.1038/nmeth.2808.

714 Figures and captions

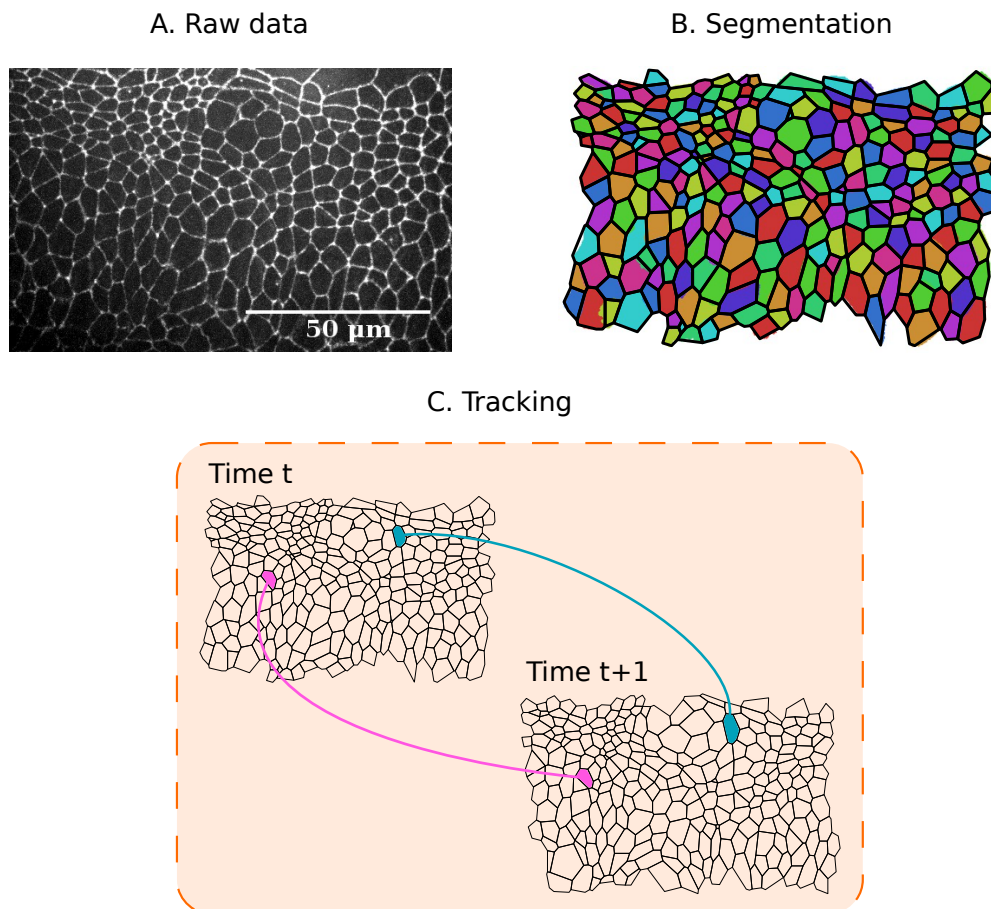


Figure 1: Pipeline for analysing epithelial tissues. (A) Example raw data. Frame of a live-imaging microscopy video of the lateral epidermis of a stage-11 *Drosophila* embryo, expressing DE-Cadherin::GFP. See Experimental Methods for details. (B) Segmentation of this image, showing cell shapes (coloured regions) and polygonal approximation based on three-cell junctions (black lines). See Methods section for details of segmentation. (C) Cell tracking involves registering individual cells across consecutive segmented images.

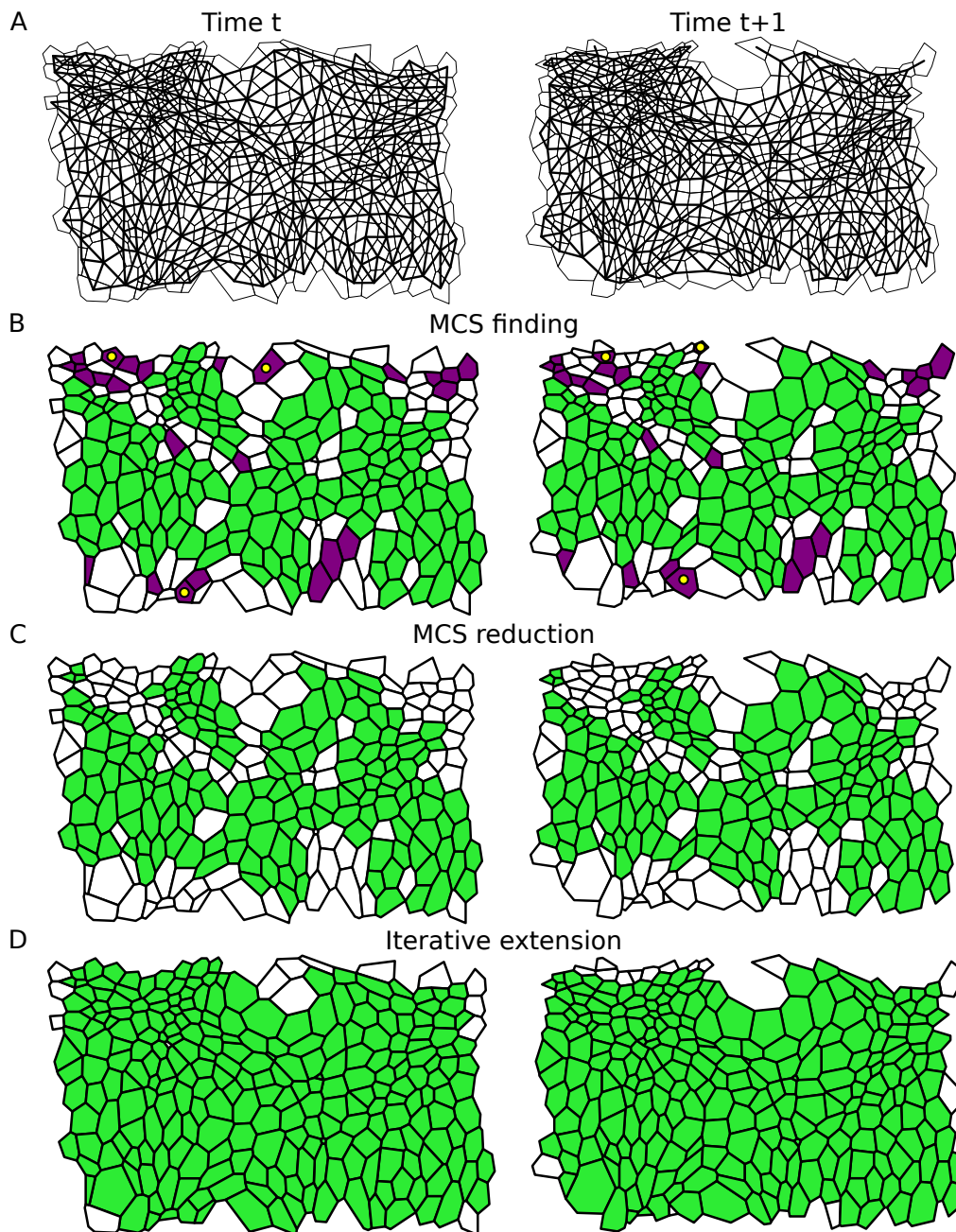


Figure 2: Illustration of our cell tracking algorithm. (A) Grey: Two consecutive segmented time-lapse images (left and right columns) of the lateral epidermis of a stage-11 *Drosophila* embryo, taken five minutes apart. See Experimental Methods for details. There are several cell neighbour exchanges between these images. Black: Overlay of the network of cells that the algorithm uses for cell tracking. Cells in the tessellation correspond to network vertices that are connected by an edge if the cells are adjacent. (B) We first identify a cell mapping between the two graphs based on the MCS. This includes correctly tracked (green/light) cells and cells that have only few tracked neighbours (purple/dark). Here, the MCS incorrectly tracks three cells (yellow/light dots). (C) Weakly connected cells and small isolated clusters of cells are removed from the MCS to prevent mismatches. (D) An extended tracking mapping is constructed, which includes the maximum possible number of cells. See Methods section for details. The remaining white cells have entered or left frame of view between images and therefore are not tracked.

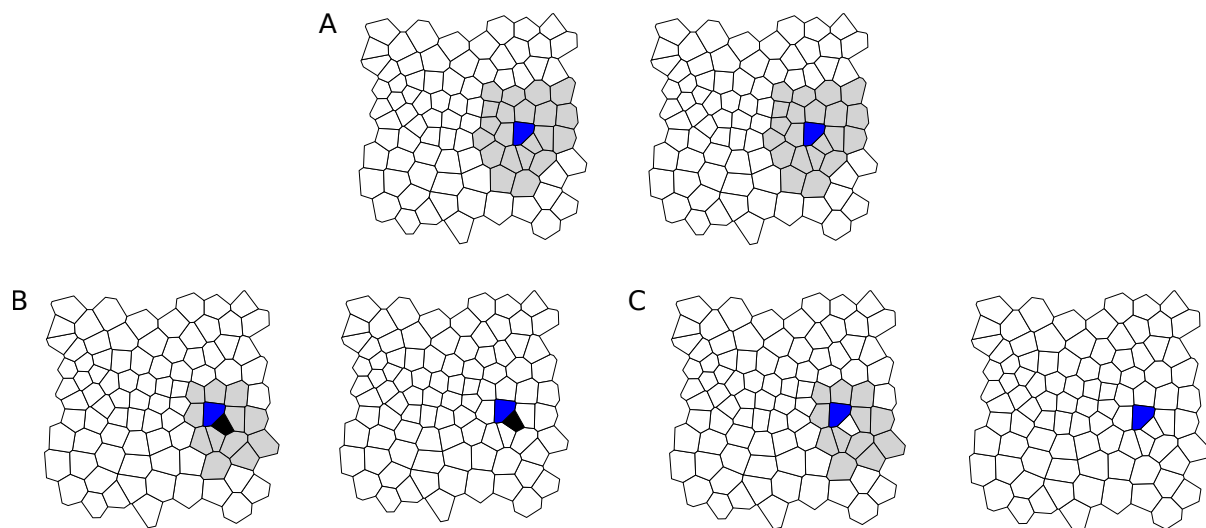


Figure 3: Construction of the MCS. (A) The algorithm picks a first match of cells for the MCS (blue) if their neighbourhoods form identical networks. The considered neighbourhood (grey) includes all neighbours and second nearest neighbours. (B-C) Additional cells are added to the MCS iteratively by inspecting the MCS between the grey area on the left, and the white area on the right. In (B), where the black cell is paired correctly, the local MCS is larger than in (C), where the selected cell is not considered for mapping. Hence, the pairing of black cells is added to the MCS.

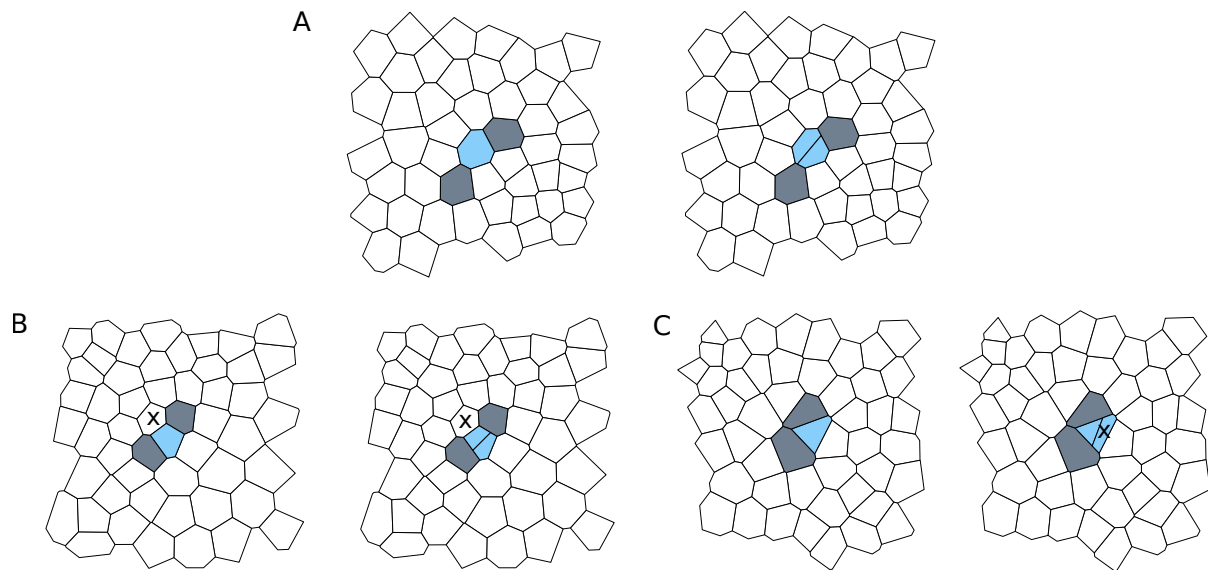


Figure 4: Resolving division events. Dividing cells are coloured blue. (A) Division events are resolved by identifying cells that gain an edge between the time frames (grey). The dividing cell and the daughter cells are shared neighbours of such cells. (B) When one of the daughter cells is four-sided, two mother cells are possible, the blue marked mother cell, and the cell marked by an 'x'. (C) If one of the daughter cells is three-sided, the mother cell can be mistaken for having gained an edge if it is identified with the daughter cell labelled 'x'. Our algorithm correctly resolves each of the types of division events shown in (A)-(C).

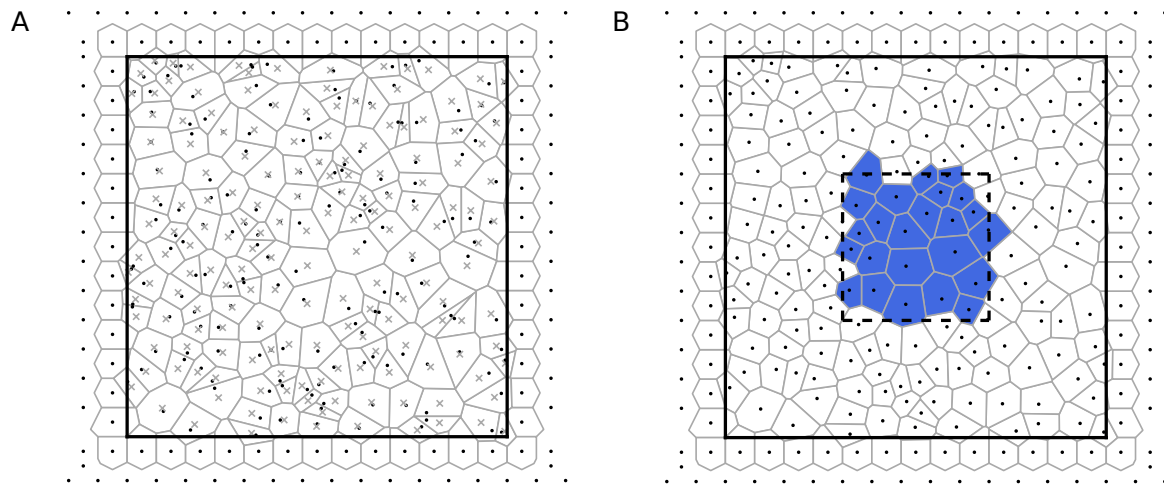


Figure 5: Generation of *in silico* data. (A) Random seeds (black dots) are placed inside a domain Ω (black line). Additional seeds are placed outside Ω . The Voronoi tessellation of all seeds is shown in grey, excluding Voronoi regions corresponding to the outermost row of seeds, since these are large or unbounded. The centroids of the Voronoi regions (grey crosses) differ from the seeds. (B) The centroids of the Voronoi regions in (A) are used as seeds for a new Voronoi tessellation, for which evenly spaced seeds are again added outside the domain Ω . Voronoi regions whose centroids lie within a central rectangle (dashed black line) are collected to form the *in silico* tissue (blue). In this figure, one Lloyd's relaxation step is shown. Throughout this study, we generate *in silico* tissues using four Lloyd's relaxation steps.

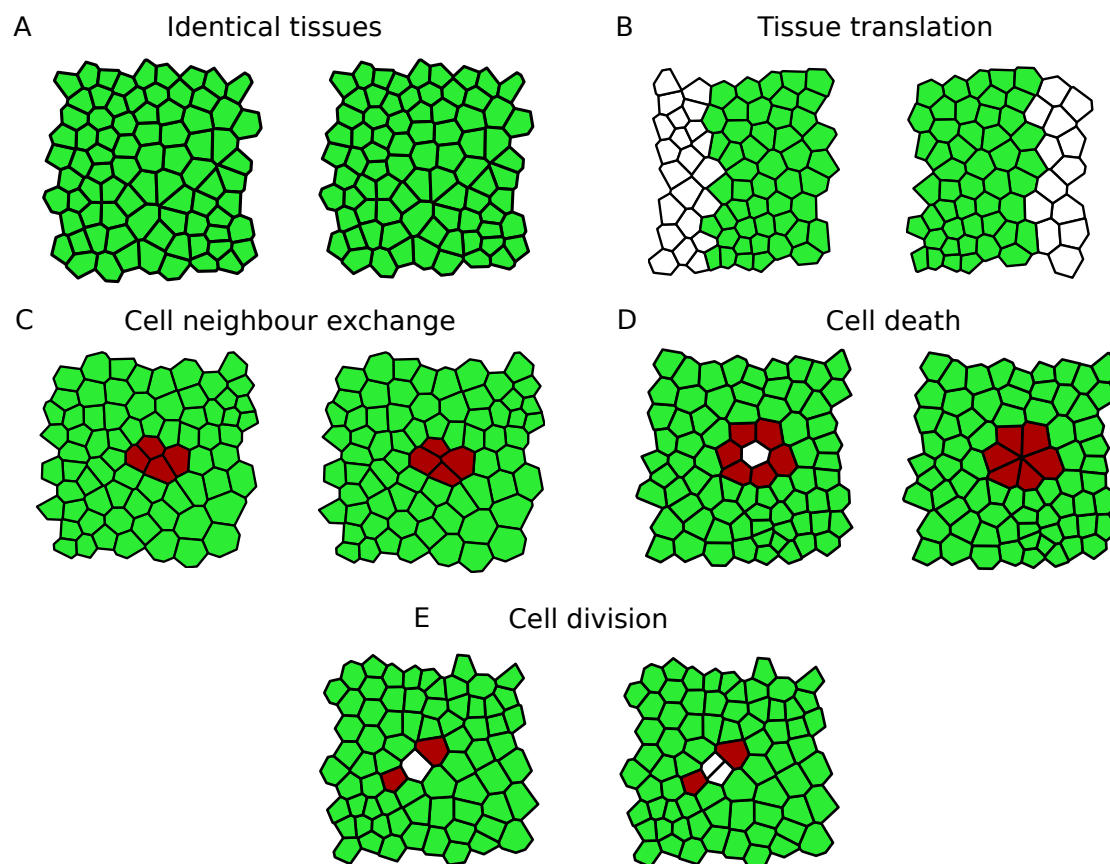


Figure 6: Examples of *in silico* test cases. In each image, cells identified by the MCS algorithm are highlighted in green (light), whereas cells that have been filled in by the post-processing steps are highlighted in red (dark). The algorithm tracks cells between identical tissues (A), in tissues undergoing translation (B), cell neighbour exchange (T1 transition) (C), cell removal (D) and cell division (E).

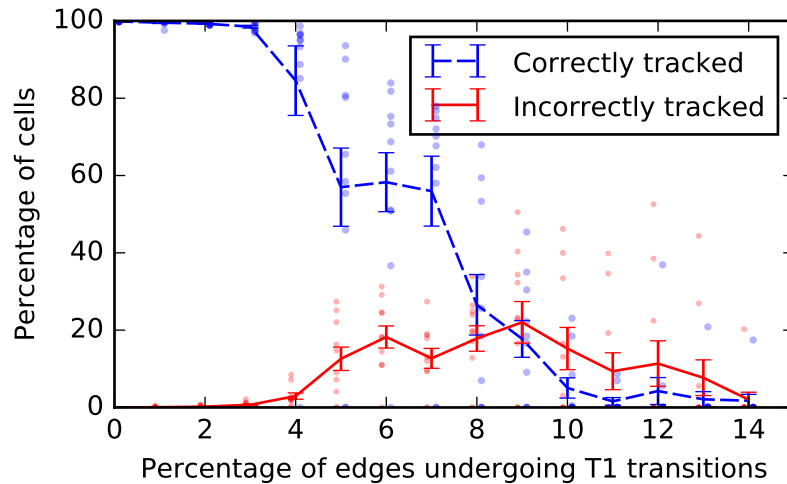


Figure 7: Success rate of the algorithm for *in silico* tissues with increasing frequency of cell rearrangement. Virtual tissues spanning 20 cell lengths in each dimension are generated, and T1 transitions are applied to an increasing proportion of the inner edges of the tissue. For each ratio of T1 transitions, 10 repetitions of the test are run, and the ratio of correctly and incorrectly tracked cells in the tissue is recorded. The dashed blue and solid red lines correspond to mean values of correctly and incorrectly tracked cells, respectively. Error bars denote the standard deviation of the mean, and results of individual runs of the test are represented by dots. When 3% of the edges in the tissue undergo T1 transitions, roughly 25% of the cells exchange neighbours.

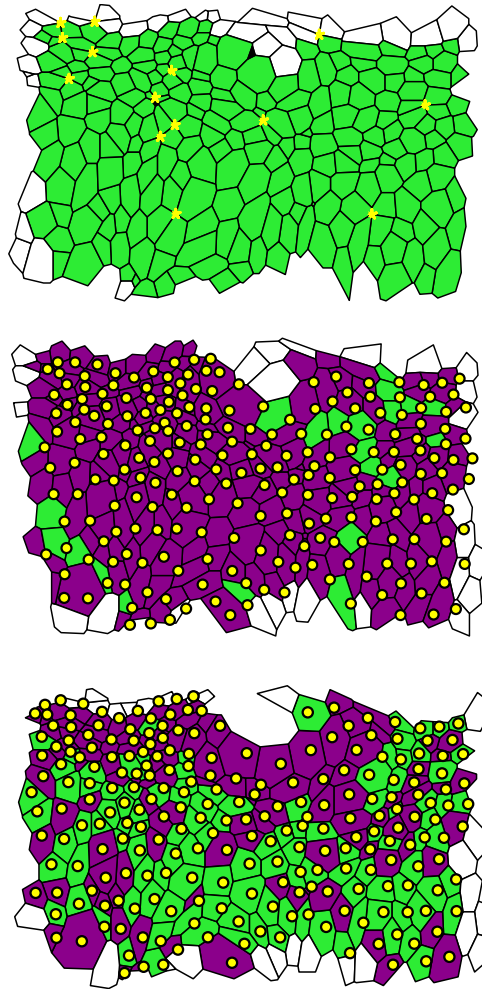


Figure 8: Three segmented data frames of an *in vivo* time-lapse microscopy video of the lateral epidermis of a stage-11 *Drosophila* embryo. Cells that are tracked across all frames are coloured green or purple, and cells that leave or enter the tissue at the boundary are white. Dying cells are black. The centroids of tracked cells of the respective previous frames are included as yellow dots, and cells that contain only their centroid from the previous frame are coloured green, whereas cells that do not contain their centroid from the previous frame, and cells that contain multiple centroids, are coloured purple. Together, the centroids and the colouring illustrate that it is challenging to track cells between the data frames using solely centroid positions. Yellow asterisks in the first frame denote higher-order junctions where more than three cells meet.

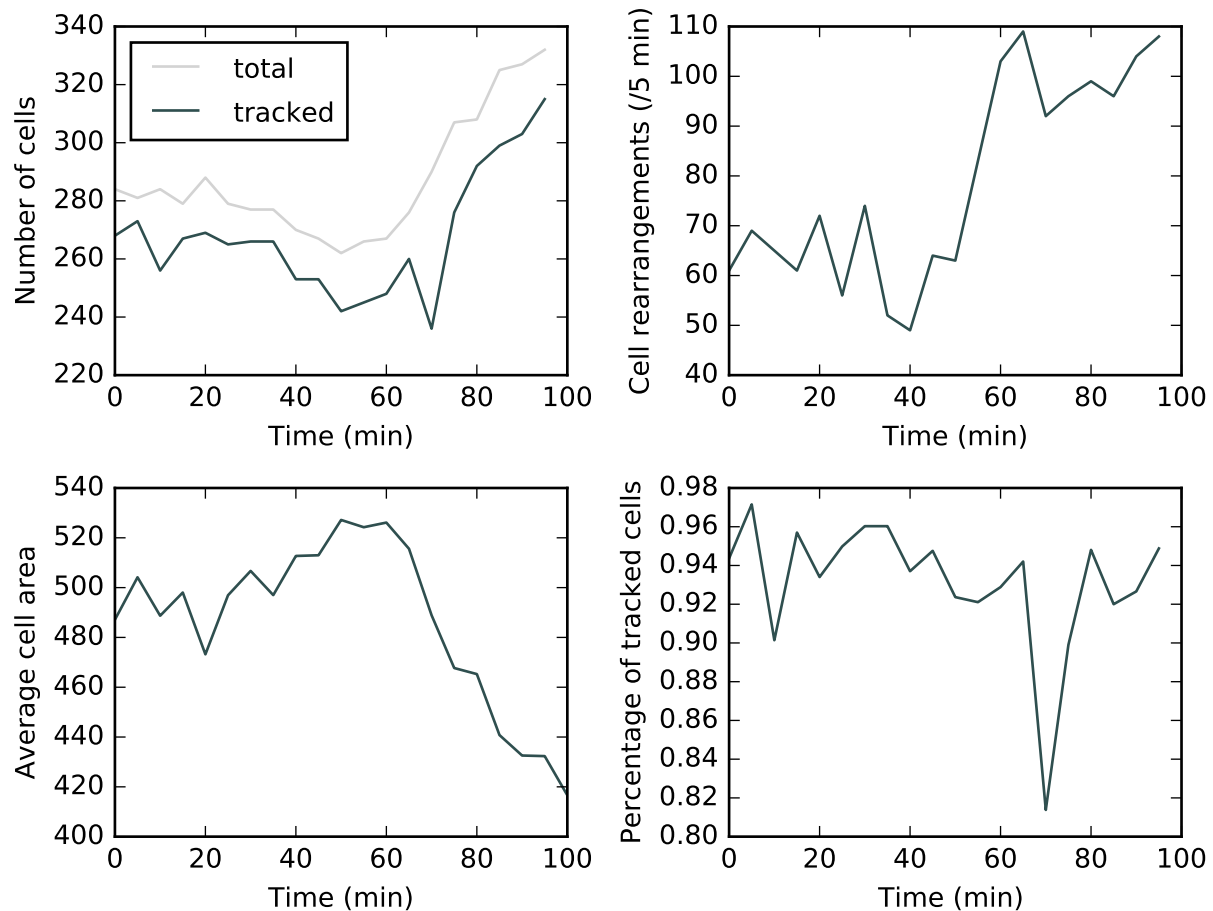


Figure 9: Tracking statistics of the *in vivo* dataset. (Top left) The total number of cells at each time point is constant initially and increases from 60 min onwards. The total number of tracked cells correlates with the total number of cells in the tissue. (Top right) The total number of rearrangements between successive time frames is measured by our algorithm. We record the total number of rearrangements as the total number of cells that either gain or lose neighbours between successive frames. (Bottom left) The average cell area in each frame decreases as the total number of cells increases. (Bottom right) The percentage of tracked cells decreases at around 70 min, when the amounts of cell rearrangement and division are highest.

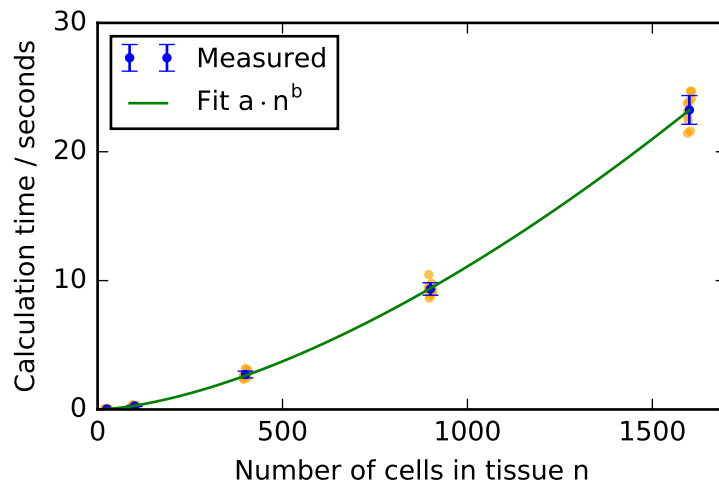


Figure 10: Scaling of the calculation times with tissue size. Square virtual tissues of varying sizes were generated and the calculation times of the algorithm under the permutation test in figure 6A recorded. Orange dots represent calculation times for individual realisations of the test and error bars denote the standard deviation. The exponent b of the polynomial fit is 1.6. The calculation times were measured on a desktop computer with an Intel i5-6500T CPU (2.5GHz) and 8GB RAM.

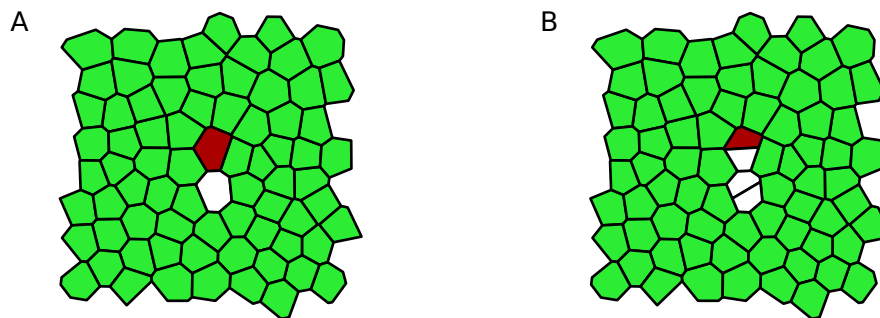


Figure 11: Tracking errors can occur if adjacent cells divide. Here, all green (light) cells are tracked correctly. One of the mother cells (red/dark) of the division events has been incorrectly associated with one of the daughter cells of the division.