Surface-guided computing to analyze subcellular morphology and membrane-associated signals in 3D

Authors

Felix Y. Zhou[1,2], Andrew Weems[1,2], Gabriel M. Gihana[1,2], Bingying Chen[1,2], Bo-Jui Chang[1,2], Meghan Driscoll[1,2,3] and Gaudenz Danuser[1,2]

Affiliation

[1]Lyda Hill Department of Bioinformatics, University of Texas Southwestern Medical Center, Dallas, TX, USA.

[2]Cecil H. & Ida Green Center for System Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA

[3]Current address: Department of Pharmacology, University of Minnesota, Minneapolis, MN, USA

Correspondence

Correspondence to Felix Zhou or Gaudenz Danuser: {felix.zhou, gaudenz.danuser}@utsouthwestern.edu

Abstract

Signal transduction and cell function are governed by the spatiotemporal organization of membrane-associated molecules. Despite significant advances in visualizing molecular distributions by 3D light microscopy, cell biologists still have limited quantitative understanding of the processes implicated in the regulation of molecular signals at the whole cell scale. In particular, complex and transient cell surface morphologies challenge the complete sampling of cell geometry, membrane-associated molecular concentration and activity and the computing of meaningful parameters such as the cofluctuation between morphology and signals. Here, we introduce u-Unwrap3D, a framework to remap arbitrarily complex 3D cell surfaces and membrane-associated signals into equivalent lower dimensional representations. The mappings are bidirectional, allowing the application of image processing operations in the data representation best suited for the task and to subsequently present the results in any of the other representations, including the original 3D cell surface. Leveraging this surface-guided computing paradigm, we track segmented surface motifs in 2D to quantify the recruitment of Septin polymers by blebbing events; we quantify actin enrichment in peripheral ruffles; and we measure the speed of ruffle movement along topographically complex cell surfaces. Thus, u-Unwrap3D provides access to spatiotemporal analyses of cell biological parameters on unconstrained 3D surface geometries and signals.

Main

Advances in 3D high-resolution live-cell microscopy and biosensor design enable integrative studies of the dynamic interplay and causal relations between cell morphology and signal transduction *in vitro* and *in vivo*. By reshaping the plasma membrane into diverse morphologies, cells sense, respond to and remodel their local environment[1-6]. Many cell types adopt shapes that are tailored to their characteristic function[7-11]. Cell morphology has thus long been recognised as a proxy of cell state and as a marker of differentiation[9,11,12]. Mechanically, the plasma membrane integrates internal and external forces, which affects cell fate through mechanotransductive proteins and changes in cytoskeleton and nuclear morphology[13-15]. Structurally, the plasma membrane serves as a platform for catalysing chemical reactions[16-20] and as a spatiotemporal organiser of signalling activity through the creation of binding sites, local confinements and molecular concentration in scaffolds, diffusion traps, and by phase separation[16,21-23]. These reactions occur locally at the nanometer or micron length scale or in global bursts that span the entire cell[18,24]. Understanding the salient biophysical processes that govern the formation and persistence of these subcellular signalling domains and how these domains regulate biochemical signal transduction remains enigmatic. Systematic identification of this intricate regulatory interplay between cell shape and molecular signalling necessitates consistent temporal tracking of the local 3D cell geometry and conjoint sampling of the corresponding membrane-associated molecular concentration and activity.

Cell surfaces are extracted from binary segmented image volumes and stored as a mesh, a data structure described by a list of the Cartesian 3D vertex coordinates on the surface and a second list specifying how the individual vertices are connected into triangles or faces. Tracking the correspondence between two 3D surface meshes is an active area of research in computer graphics[25-28] and none of the methods have been adopted to cell imaging. A particular technical challenge that arises when adapting techniques from computer graphics with applications to cell biology is the non-convexity, irregularity and high curvature of surface protrusions on most cell shapes. Very few methods have been proposed to accurately follow such geometries over time and have largely been demonstrated on well-defined shapes such as human pose[29] or hands[30,31]. Generally, these methods track by matching meshes from consecutive timepoints. To match meshes, methods attempt to assign a unique signature per vertex or face to establish a matching between vertices and faces by minimizing a loss metric[32,33]. However, this approach is inherently sensitive to mesh quality, uniqueness of the signature, optimizer convergence and is difficult to generalize when tracking surfaces over many timepoints. Crucially, meshes segmented from two different timepoints have different numbers of vertices and faces and the lack of the exact same surface features poses ambiguity in matching. Alternatively, individual 3D image volumes may be first registered spatiotemporally before mesh extraction, as is done in neuroscience[34-37]. This approach is robust to mesh quality and sampling errors, however deformations must be small between timepoints. For example, long or thin cell surface structures such as lamellipodia and filopodia suffer voxel undersampling, limiting the registration to cell surfaces with largely globular features such as blebs[38]. This problem could be remedied by mapping the 3D surface to the unit sphere[39-41]. Indeed, in macroscopic imaging application, this procedure has enabled registration of complex geometrical features such as brain folds directly on the 3D sphere or in derivative 2D unwrapped images[27,42-44]. Unfortunately, this strategy requires closed surface topologies with no holes (genus-0 surfaces), which is not generally guaranteed in live-cell microscopy. Alternatively, one can selectively segment surface motifs and track these in 3D whilst mapping surface-proximal molecular signal intensity[38,45-47]. Like the 3D surface tracking, this

78 approach is also susceptible to the variable quality of the segmented motifs used for matching and non-
79 convexities of the surface.

81 Here, we develop a general and comprehensive software solution, u-Unwrap3D, for surface-guided
82 computing. u-Unwrap3D remaps arbitrarily complex 3D subcellular morphology and membrane associated
83 signals to equivalent lower dimensional representations that allow for optimized computation of surface
84 features and spatiotemporal tracking and sampling of cell geometry and associated molecular entities. We
85 demonstrate the power of this approach in applications to i) the unsupervised segmentation of diverse surface
86 motifs; ii) the quantification of septin polymer recruitment to dynamic cell surface blebs and iii) the
87 measurement of travel speed of actin-enriched surface ruffles.

Results

u-Unwrap3D for surface-guided computing

92   Given an input Cartesian 3D surface $S(x, y, z)$ as a 3D mesh, u-Unwrap3D computes a series of equivalent
93   surface representations (Fig. 1a, Suppl. Video 1). The input surface $S(x, y, z)$ is smoothened to find a genus-
94   0 reference surface $S_{\text{ref}}(x, y, z)$ and then mapped to the sphere, $S^2(x, y, z)$, unwrapped into a 2D $S_{\text{ref}}(u, v)$
95   using UV-unwrapping and the (u, v) parameterized $S_{\text{ref}}(x, y, z)$ propagated along the steepest gradient of its
96   signed distance transform to construct a topographic representation of the input surface, $S(d, u, v)$. The
97   mappings between representations are bijective and constructed to minimize the associated conformal
98   (preservation of aspect ratio) and equiareal (preservation of surface area fraction) errors[48] (Extended Fig.
99   1a,b). We denote $S(\cdot)$ and $V(\cdot)$ as surfaces and volumes, respectively, relative to a coordinate system
100 indicated in brackets. The variables $F_i\big(S(\cdot)\big)$ and $I_i\big(V(\cdot)\big)$ denote surface- and volume- associated signals of
101 interest. These signals may describe geometrical quantities, (like mean curvature, *H*), integer labels (like
102 segmented surface protrusions) or molecular activities (like molecular concentrations or activities). Thus, u-
103 Unwrap3D provides a framework to map these variables between different surface representations, each of
104 which is suited for different computational tasks.

106 **Step 1** iteratively smoothens out salient surface features on $S(x, y, z)$ using conformalized mean curvature
107 flow (cMCF)[49] to determine a genus-0 reference surface $S_{\text{ref}}(x, y, z)$ without holes or 'handles'. The term
108 'handle' refers to the holes in a loop mesh structure such as the handle of a teacup that unlike 'holes' does
109 not involve missing/incomplete surface patches in a mesh. cMCF iteratively displaces vertices with a speed
110 proportional to the mean curvature at each vertex (Extended Fig. 1c). The input surface is thereby
111 preferentially deformed into the largest inscribable sphere. In the absence of *a priori* markers for the reference
112 shape such as cell cortex markers, the rate of decrease in mean absolute Gaussian curvature K is monitored
113 to determine a stopping iteration (Methods). The Gaussian curvature K is a shape-invariant measure of local
114 curvature. Accordingly, the same shape (e.g. a sphere) has identical K value irrespective of size[50]. K is thus
115 well-suited as a criterion to terminate the cMCF iterations. cMCF is agnostic to minor mesh imperfections
116 such as small holes and handles but does not change the genus. Any holes or handles in the input surface
117 are still present. However, because of the smoothing, these holes are more regular and smaller. Still in Step
118 1, we compute a genus-0 mesh of the reference surface $S_{\text{ref}}(x, y, z)$ through filling all holes in the volume
119 enclosed by the intermediary cMCF-processed surface and remeshing of the resulting body (Methods). The
120 remeshing changes the vertex position and face topology. To restore bijectivity between the input surface
121 $S(x, y, z)$ and $S_{\text{ref}}(x, y, z)$ we match the mesh $S_{\text{ref}}(x, y, z)$ with the mesh of the intermediary cMCF-processed
122 surface that is bijective to $S(x, y, z)$, (Methods). Any associated measurements $F_i(S(x, y, z))$ are mapped to
123 $F_i(S_{\text{ref}}(x, y, z))$ by interpolation. In **Step 2**, the genus-0 reference surface, $(S_{\text{ref}}(x, y, z)$ is quasi-conformally
124 mapped to the unit sphere without folds[39,51] (Extended Fig. 1d). This spherical parametrization is denoted
125 $S_Q^2(x, y, z)$. Per the uniformization theorem, such a mapping always exists for a genus-0 surface[52-55]. The
126 quasi-conformal spherical parameterization $S_Q^2(x, y, z)$ severely shrinks surface extremities deviating from the
127 sphere[27], even for roughly globular shapes (Extended Fig. 1d). Consequently, surface features with high
128 curvature are undersampled and disproportionately represented relative to their original Cartesian 3D surface
129 area. This can detrimentally affect downstream analyses such as segmentation and tracking[27]. To mitigate
130 this problem, we iteratively diffuse in **Step 3** the area distortion factor per face by advecting vertex positions
131 on the sphere[27] at the expense of increased conformal error (Extended Fig. 1e). In **Step 4,** this quasi-
132 equiareal sphere $S_\Omega^2(x, y, z)$ is bijectively unwrapped to the 2D plane, $S(u, v)$, using equirectangular projection,
133 in short UV-mapping, with (u, v) denoting the spherical polar and azimuthal angles, respectively. UV-
134 mapping introduces the strongest distortions to signals at the north and south poles of the sphere. To visualize
135 features of interest with minimal distortion, u-Unwrap3D optionally infers a rotation matrix based a

136 weighted principal component analysis of surface variables, such as the local curvature (Extended Fig. 1f,g,
137 Methods). If the input Cartesian 3D surface mesh $S(x,y,z)$ is genus-0, the generation of a reference surface,
138 $S_{\mathrm{ref}}(x,y,z)$ may be skipped and 2D equiareal surface unwrapping realised directly (Fig. 1b, Suppl. Video 2).
139 We note that an input genus-X Cartesian 3D surface $S(x,y,z)$ is also directly unwrapped into 2D through
140 steps 1-4 of u-Unwrap3D, but not in an equiareal manner (Extended Fig. 1h). In **Step 5**, the first part remaps
141 the Cartesian 3D volume $V(x,y,z)$ and associated signals $I_i(V(x,y,z))$ into a topographic volume $V(d,u,v)$
142 coordinate system that is normal to the reference surface, $S_{\mathrm{ref}}(x,y,z)$. The second part establishes a bijective
143 mapping of (d,u,v) to (x,y,z) coordinates, $S_{\mathrm{ref}}(u,v)$, i.e. the (u,v) parameterized reference surface of
144 $S_{\mathrm{ref}}(x,y,z)$, is propagated in Cartesian 3D space in the surface normal direction at equidistant steps of α
145 voxels along the steepest gradient of the signed distance function, $\nabla\Phi(x,y,z)$, for a total of D steps.
146 Interpolation of the respective Cartesian volumetric signal intensities, $I_i(V(x,y,z)$ at the (x,y,z) coordinates
147 indexed by (d,u,v) generates the topographic 3D volume equivalents, $I_i(V(d,u,v))$. Finally, in **Step 6** the
148 topographic 3D surface representation, $S(d,u,v)$ of the input surface $S(x,y,z)$ is obtained by surface
149 meshing the topographic binary volume segmentation.

151 In summary, u-Unwrap3D generates bijective mappings of a given genus-X surface between 5 equivalent
152 surface representations; Cartesian 3D, $S(x,y,z)$, genus-0 reference 3D, $S_{\mathrm{ref}}(x,y,z)$, the unit 3D sphere,
153 $S^2(x,y,z)$, topographic 3D, $S(d,u,v)$, and the 2D plane image, $S(u,v)$ (Fig. 2a), while simultaneously
154 transforming Cartesian 3D to topographic 3D volumes (Fig. 2b). This was made possible by two crucial
155 choices; the use of cMCF and voxelization to construct a genus-0 reference surface, $S_{\mathrm{ref}}(x,y,z)$ to realise
156 spherical parameterization (Step 1) and the implementation of an efficient numerical scheme to relax area
157 distortion on the 3D sphere (Step 3). The former allows us to construct $S_{\mathrm{ref}}(x,y,z)$ as a proxy of the genus-
158 X $S(x,y,z)$ surface mesh and to unwrap this 3D surface into one 2D (u,v) image, instead of requiring multiple
159 2D (u,v) images, which simplifies downstream analysis[56-59]. The latter ensures that the unwrapped 2D (u,v)
160 image captures the salient surface features of $S_{\mathrm{ref}}(x,y,z)$, and by extension the genus-X $S(x,y,z)$ surface.
161 Importantly, the bijectivity of the mappings guarantees that for any point on any of the surface or volume
162 representations matching points exist on any of the other surfaces or volumes. Moreover, the bijectivity
163 guarantees preservation of the point topology, i.e. a series of points ordered in clockwise fashion on one
164 surface representation maps to a series of points ordered in the same way on any of the other surface
165 representations and preserves the local neighbourhood relationships. As a result, we can apply mathematical
166 operations defined in any one of the representations and map the results to any other. u-Unwrap3D thus
167 supports the optimal spatiotemporal analysis of unconstrained surface geometries and associated signals.

Validation of u-Unwrap3D on diverse surface motifs

170 We validated the generality and performance of u-Unwrap3D by application to 66 single cell images acquired
171 by high-resolution light sheet imaging[45,60]. The dataset span morphologically diverse cells with blebs,
172 lamellipodia and filopodia. The cell surfaces were meshed with marching cubes and segmented within the u-
173 Shape3D software[45]. Small errors in the initial segmentation and meshing process cause high-order genus
174 surfaces with topological holes and handles, which cannot be unwrapped directly (Extended Fig. 2a). Holes
175 can also generate non-watertight surface meshes – surfaces that are not closed and have no clearly defined
176 inside volume[48,61] possessing potentially complex internal volumetric structures that violate the assumptions
177 of standard 3D mesh processing algorithms.

179 We first tested the number of input cell surfaces for which u-Unwrap3D could successfully run all steps 1-6
180 and compute all 5 of the representations as a measure of generality and robustness. Notably only 6/66 (11%)
181 input cell surfaces were genus-0 and only 36/66 (55%) were watertight (Extended Fig 2b). In 63/66 cases,
182 (>95%) we successfully ran all steps and obtained all representations (Extended Fig 2b). The three failures
183 occurred in scenarios, in which the holes and handles remaining after the application of cMCF were still too
184 large for the volume dilation to generate a genus-0 reference surface after remeshing (Fig. 1b, Step 1)
185 (Extended Fig. 2c). In all successful cases, cMCF and binary voxelization under volume dilation generated
186 genus-0 reference surfaces within a median of 10 iterations (Extended Fig 2c, c.f. lamellipodia). Fig. 2c
187 shows extracted representations for challenging examples with blebs, lamellipodia and filopodia (Suppl.
188 Video 3-5).

190 We next tested the robustness and performance of the $S_{\mathrm{ref}}(x,y,z)$ spherical parameterizations, (Fig. 1b,
191 Steps 2-3). Extended Fig. 3a confirms that the quasi-conformal spherical parameterization (Step 2) minimizes
192 the conformal error to the ideal value of 1, with the largest error in cells with filopodia (1.016 ± 0.013). We also
193 verified the need to relax local area distortion. Whilst quasi-conformal spherical parameterization $S_\Omega^2(x,y,z)$
194 is equiareal for blebs (0.978 ± 0.037), the median area distortion showed that the surface fraction of
195 lamellipodia was down to 0.432 and in filopodia to just 0.140 with respect to their original area fraction on the

reference 3D, $S_{\mathrm{ref}}(x, y, z)$ surface, let alone $S(x, y, z)$. Our scheme for area distortion relaxation (Methods) produces a quasi-equiareal spherical parameterization (Step 3) in blebs (0.985±0.012), and successfully achieves the ideal value of 1 in lamellipodia (1.000±0.000) and filopodia(1.000±0.001), within a maximum median of 23 iterations for lamellipodia (Extended Fig.3b, Table i). We further tested the ability of our relaxation scheme to balance the trade-off between the two extremes of conformal to equiareal spherical parameterizations using different stopping criteria (Extended Fig.3b, Table ii-iv). The initial parameterization without any area-distortion relaxation (iteration 0) is by design conformal but also found to satisfy the most isometric parameterization (MIP)[62]. Running for $t_{\Omega} <$ a maximum of 50 iterations yields an equiareal parameterization for all motifs. At $t \approx \frac{1}{2} t_{\Omega}$ iterations the relaxed mesh jointly minimizes the summation ($Q + \ln \lambda$, Methods) of conformal (Q) and area distortion (λ) errors. At $t \lesssim t_{\Omega}$ iterations the relaxed mesh is the area-preserving MIP[63]. As expected, this latter parameterization does not fully minimize area distortion (blebs (0.997±0.011), lamellipodia (0.979±0.005) and filopodia (0.980±0.028)) but exhibits slightly lower conformal errors and consequently higher quality faces than a pure equiareal mapping.

Lastly, we tested how accurately $S_{\mathrm{topo}}(x, y, z)$, which defines the topographic 3D mesh, $S(d, u, v)$ (step 5) mapped back into Cartesian coordinates reconstructs the input surface, $S(x, y, z)$. For all cells, $S(d, u, v)$ was computed with a (u, v) image grid size of 1024x512 pixels. The aspect ratio, $2N$ x N ($N = 512$) was chosen to preserve the ratio between the equatorial circumference and the length of the arc between north and south poles of a sphere. Compared to the input surface $S(x, y, z)$, $S(d, u, v)$ is lower genus and provides higher face quality (Extended Fig. 3c). We assessed the discrepancy between $S_{\mathrm{topo}}(x, y, z)$ and $S(x, y, z)$ using 4 metrics; Chamfer distance (CD), sliced Wasserstein distance (SW_1)[64], and differences in total surface area (ΔA) and volume (ΔV) (Extended Fig. 3d, Methods). Considering inevitable rasterization errors when mapping the floating-point precision 3D sphere $S_{\Omega}^2(x, y, z)$ to $S(u, v)$ defined on an integer (u, v) image grid, we measured low vertex position errors according to CD and SW_1. Cells with lamellipodia had the lowest error (median CD=1.77 voxel, SW_1=0.93 voxel) and cells with blebs were slightly worse (median CD=2.79 voxel, SW_1=4.28 voxel), likely due to their intrinsically small height (small topographic d). As one would expect, cells with long, thin filopodia displayed the largest discrepancies (median CD=10.33 voxel, SW_1 =18.45 voxel). Correspondingly we measured a small ΔA (+1.2%) and ΔV (+7.9%) for cells with lamellipodia. ΔA was larger for cells with blebs (-11.6%) and measured to be too large for filopodia (-55.3%) when compared to ΔV differences measured after making $S(x, y, z)$ watertight (+4.2% blebs, +3.4% filopodia). Visualization of exemplar cells show good geometric correspondence between $S_{\mathrm{topo}}(x, y, z)$ and $S(x, y, z)$ (Extended Fig. 3e). Salient surface features were largely captured, albeit smoothened and blurred in $S_{\mathrm{topo}}(x, y, z)$ when local surface regions were underrepresented due to being distant relative to $S_{\mathrm{ref}}(x, y, z)$ (Extended Fig. 3e, black triangles, 1st row blebs and 4th row lamellipodia). Most of the primary morphological features, namely the length and thickness of long, thin filopodia (Extended Fig. 3e, green triangles), except those located both densely together and distant relative to $S_{\mathrm{ref}}(x, y, z)$ (Extended Fig. 3e, red triangles), were captured. u-Unwrap3D was able to capture both the complex lamellipodia folds and curved cell bodies to high accuracy (Extended Fig. 3e, row 2,4). Closer inspection of $S_{\mathrm{topo}}(x, y, z)$ and $S(x, y, z)$ in these cells traced a large ΔA to meshing errors in the input surface $S(x, y, z)$, which caused internal volumetric structures to be merged into the cell surface representation, and overestimation of total surface area. These errors affect the CD and SW_1 to lesser extent.

In summary, our results demonstrate that u-Unwrap3D is robust and applicable to process unconstrained geometries. For maximum resolution of high curvature surface features, a genus-0 reference surface $S_{\mathrm{ref}}(x, y, z)$ proximal to the input surface $S(x, y, z)$ is recommended with a large (u, v) grid size N and small α step sizes when propagating $S_{\mathrm{ref}}(u, v)$. However, these choices depend on the quality of the input surface mesh $S(x, y, z)$, which depends on the robustness of cell segmentation in the face of noisy image raw data.

u-Unwrap3D enables unsupervised instance segmentation of subcellular surface motifs

The unbiased identification and segmentation of individual protrusive features in unconstrained 3D surface geometries is nontrivial. Cellular protrusions present complex morphological characteristics that are difficult to define descriptively. Even well-known morphological motifs exhibit significant heterogeneity and ambiguity. Not all blebs are spherical, lamellipodia are often plate-like with high curvature ridges but otherwise have no readily-defined shape prior, and filopodia, though long and thin, can sprout haphazardly from elevated 'stumps' or even off of each other. In areas of dynamic and dense protrusions, where does one protrusion start and another end? Consequently, most existing approaches focus on particular surface features of interest such as 'ridge' networks that can be segmented by designed imaging filters or through trained semantic segmentation, with morphological processing and parameter tuning[46,47,65,66]. With u-Shape3D we

introduced a multi-class morphological motif detection by partitioning the 3D surface into convex patches and applying support vector machines trained with expert annotation to classify the patches into pre-specified motif types[45]. However, this approach cannot detect and segment all protrusions generally, only the limited motifs for which the supervised classifier has been trained on. Lastly, even after obtaining the segmentations, how do we systematically measure salient protrusion properties? For example, with respect to what reference surface should protrusion height be measured? Where is the protrusion width to be measured? How is the internal volume of a protrusion determined?

These segmentation and characterization problems can be significantly better defined in the topographic 3D surface representation $S(d, u, v)$, which captures in one field-of-view all surface features protruding normally to $S_{\text{ref}}(x, y, z)$. As d preserves the total Cartesian 3D curvilinear distance from $S(x, y, z)$ to $S_{\text{ref}}(x, y, z)$ along the gradient of steepest descent we can formally define a 'protrusive' feature as having a d-coordinate greater than that of a reference topographic surface, $S_{\text{ref}}(d_{\text{ref}}, u, v)$, and measure the protrusion height as the difference, $h = d - d_{\text{ref}}$. For example, protrusive features could be specified as those having $h > \bar{h}^{cMCF}$, the mean height of $S(d, u, v)$ relative to the planar topographic 3D cMCF surface $S_{\text{ref}}^{\text{cMCF}}(d, u, v)$ (i.e. $d_{\text{ref}} = f(u, v) = 0$). However, this definition leads to under-segmentation (Extended Fig. 4a). A remedy would be an intermediate surface $S_{\text{ref}}(d_{\text{ref}} = f_{\text{smooth}}(u, v), u, v)$, which interpolates between the input rugged topographic cell surface, $S(d, u, v)$ and the 2D planar cMCF cell surface $S_{\text{ref}}^{\text{cMCF}}(d, u, v)$, (Fig. 3a,b). Whereas this problem is difficult to frame in Cartesian 3D, in the topographic space the interpolation can be solved naturally by using asymmetric least squares (ALS) optimization with a Whittaker smoother[67,68], where the asymmetric weights allow us to account for the heterogeneous protrusion height; and the desired level of surface smoothness can be incorporated as a regularization term (Extended Fig. 4b, Methods). To use ALS, we create a (u, v)-parameterized approximation of $S(d, u, v)$ with $d \simeq f(u, v)$ using straightforward image processing procedures (Extended Fig. 4b, Methods). By exploiting these properties of the topography space, we developed a general approach to segment any protrusion motif including blebs, lamellipodia and filopodia (Extended Fig. 4c,d) with minimal heuristic parameters to tune. Importantly, we did not need to design specialized image filters[65,66], compute and cluster feature descriptors[46,69-71], or require data training[45-47].

We demonstrate the segmentation of individual protrusion instances, capturing motifs identified by uShape3D, but without the need for training annotations. By construction, in topographic 3D (d, u, v) space all surface protrusions are oriented upwards with increasing d. Moreover, the tops of protrusions are individually separated as local regions of high topographic mean curvature, which we identify by thresholding and applying connected component labelling in the topographic volume, $V(d, u, v)$ (Fig. 3c). Mapping the segmented regions back onto the topographic mesh $S(d, u, v)$, we diffuse these initial 'seed' labels across the surface using a combined geodesic distance and dihedral angle affinity matrix to naturally segment the 'stem' of the individual protrusions (Methods). The dihedral angle measures the discontinuity in local mean curvature. It incorporates the prior intuition that the boundaries of a label should expand faster on local surfaces of homogeneous curvature such as that on a 'hill', compared to another label experiencing large curvature differences in its local surface region, such as in a valley between multiple 'hills'. The combined affinity matrix thus introduces a morphology-aware competition between segmentation labels and provides a biophysical rationale for defining which surface patches belong to individual 'seed' protrusions. Furthermore, the dihedral angle is large between a protrusion and the main cortical cell surface and thus serves as a soft stopping criteria for diffusion (Extended Fig. 4d) in addition to applying the binary protrusion segmentation from above. Lastly, we filter out protrusions that are too small and close any small holes using the Cartesian 3D surface area. The final segmentation result qualitatively and quantitatively agrees with that obtained by supervised u-Shape3D for lamellipodia (Fig. 3d) but yields more contiguous labels and is less prone to over-segmentation (Fig. 3e). Importantly, this segmentation strategy is applicable, even when not all protrusions are equiareally represented in $S(d, u, v)$, as shown by the segmentation of the majority of blebs and filopodia in exemplar cells (Extended Fig. 4d).

Individual segmented protrusions are genus-0 open-surface 3D submeshes that can be directly mapped to the 2D plane (Fig. 3f). This allows us to further refine the segmentation, for example, by detecting and splitting under-segmented blebs by a gradient watershed algorithm (Methods). Thanks to bijectivity, the refined segmentation labels can be transferred back onto the original surface mesh (Fig. 3f,g, c.f. before and after refine, black triangles). Both the before (adjusted normalized mutual information, NMI=0.57) and the after refinement (adjusted NMI=0.54) segmentations agree with u-Shape3D. However, u-Unwrap3D segmented blebs are more complete, with more blebs of larger surface area (150 blebs in total). In contrast, u-Shape3D over-segments small blebs (742 blebs) that were found to originate from erroneous meshing of internal structures in $S(x, y, z)$ (see Extended Fig.3e). This example illustrates the potential pitfalls of identifying motifs from local surface patches only with potentially imperfect input 3D meshes. In contrast, with u-Unwrap3D any

314 surfaces internal to the cell volume are readily removed when mapped into topography as these surfaces
315 have d-coordinates less than the reference surface, $S_{\mathrm{ref}}(d_{\mathrm{ref}} = f_{\mathrm{smooth}}(u,v), u, v)$.

317 Finally, the representation $S(d, u, v)$ enables partitioning of the input cell volume into the sum of a reference
318 volume representing the underlying cortical cell body and the unique volume occupied by individual
319 protrusions (Fig. 3h). We do so by (u, v)-parameterizing the reference cortical surface submesh, $S_{\mathrm{ref}}(d, u, v)$
320 after removing all individual protrusion submeshes as a grayscale image such that the pixel intensity value
321 at (u, v) is the respective d-coordinate, and inpainting the missing d-coordinates at (u, v) coordinates
322 corresponding to the subtracted protrusions to generate a full reference binary volume (Extended Fig. 4e,
323 Methods). For the protrusions, we first devised a marker-controlled lateral watershed depth propagation to
324 diffuse the surface-based protrusion segmentation labels uniquely throughout the full topographic (d, u, v)
325 space slice-by-slice, top-to-bottom (Extended Fig. 4f). Then, individual protrusion volumes were generated
326 by masking the propagated label volume with the reference binary volume (Extended Fig. 4g). We compared
327 our topography-guided decomposition strategy to a fully Cartesian 3D mesh processing approach whereby
328 individual protrusion submeshes were first closed by constructing a surface patch that minimized the local
329 bending (or harmonic) energy[72] (Methods). The closed reference volume was then generated using all such
330 patches to impute the residual holes in the raw reference surface. Fig. 3i panels i-ii show that the computed
331 surface area (slope=0.90) and volume (slope=0.95) of individual protrusions are similar for both methods.
332 However, the 3D mesh processing protrusions consistently under-measure larger protrusions. Crucially, the
333 imputed reference volume appears artefactual. Where protrusions were located, the surface is overly smooth,
334 and even involuted. These regions contrast starkly with non-imputed surface areas between protrusions,
335 creating artificial 'peaks' and 'ridges' of high mean curvature (black arrows). In comparison, the topography-
336 guided reference volume is mechanically more plausible.

338 In summary, the ability to map freely between topographic and Cartesian 3D surfaces and their respective
339 volumetric representations enabled us to design simple and generalizable methods to detect and segment in
340 an unsupervised fashion individual morphological motifs from unconstrained surface geometries. To
341 bijectively map the topographically segmented surface protrusion labels onto the 2D plane, which is an
342 optimal representation for tracking the segmented motifs, we developed a topographic cMCF for u-Unwrap3D
343 (Fig. 3j, Suppl. Video 6, Methods).

u-Unwrap3D enables tracking of 3D subcellular surface motifs and molecular activity in 2D

346 A central goal of live cell imaging in 3D is to visualize the spatiotemporal relations between molecular activities
347 and cell behaviors, including morphodynamic outputs. Progress has been made on software developments
348 that allow unbiased and statistically meaningful analysis of cell morphology and molecular
349 distributions[45,47,60,73,74]. However, to remain algorithmically and computationally tractable these pipelines have
350 been restricted to quasi-static representations of dynamic processes. Surface-guided computing with u-
351 Unwrap3D allows us now to remedy this limitation. Dynamic behaviours on complex 3D cell shapes, including
352 their morphological and molecular signal activity changes, can be mapped to 2D representations where
353 powerful analytical pipelines exist for spatiotemporally consistent tracking. Results can be statistically
354 evaluated and, if of interest, be visualized in 3D by leveraging the bijective properties of u-Unwrap3D
355 mappings. Fig. 4 and 5 demonstrate this capacity based on two examples.

Individual blebs dynamically recruit Septins to local surface regions during retraction

358 We first analysed potential relations between dynamic surface blebbing and the recruitment of Septins. Blebs
359 are globular membrane protrusions of 1-2 µm diameter that are thought to extend in areas of localized
360 membrane detachment from the actin cortex[75,76]. Intracellular pressure expands the budding blebs outward,
361 followed by rapid assembly of a contractile actomyosin network that yields retraction. Cycles of protrusion
362 and retraction have been described to last a few tens of seconds. Associated with the cycles are molecular
363 activities both driving and responding to the morphological dynamics. One such process is the assembly of
364 Septin protein polymers at sites of negative curvature (from a cell-external perspective) emerging at the bleb
365 necks. Our previous work[38] has shown that disrupting the bleb cycle diminishes Septin assembly at the cell
366 surface. Here we now exploit the ability of u-Unwrap3D to track individual bleb cycles and quantify Septin
367 accumulation by remapping surface morphology and a fluorescent marker of Septins to an appropriate 2D
368 representation. We acquired 3D volumes of SEPT6-GFP-expressing MV3 melanoma cells every 1.2s for 200

369 timepoints. As the cortical cell body exhibits little temporal variation and blebs protrude normally to the surface,
370 the temporal mean cell surface, $\bar{S}(x, y, z)$ is a good proxy of the cell cortex. We apply u-Unwrap3D to $\bar{S}(x, y, z)$
371 to create a common static (d, u, v) coordinate space for computing topographic 3D $S(d, u, v)$ and 2D planar
372 $S(u, v)$ representations for each timepoint. $\bar{S}(x, y, z)$ was computed by meshing the mean binary volume
373 across individual binary voxelizations of the cell surface over all 200 timepoints (Methods). Note the
374 construction of a common topographic space from a single mesh for a timelapse is computationally efficient
375 but applicable only if cell shape changes lie within the Cartesian subvolume mapped by the topographic
376 space. For large shape changes u-Unwrap3D should be applied to individual timepoints and spatiotemporal
377 registration used to align surfaces to a common reference using $S_{\text{ref}}(x, y, z)$ or $S(u, v)$ representations. The
378 segmentation tools discussed above were used to detect all bleb instances from $S(d, u, v)$ and mapped to
379 $S(x, y, z)$ and $S(u, v)$ for each timepoint (Fig. 4a, Suppl. Video 7). Similarly, the computed mean curvature
380 and the normalized Septin intensity surface signals $F_i\big(S(x, y, z)\big)$ from Cartesian 3D were mapped to
381 topographic 3D $F_i\big(S(d, u, v)\big)$ and into 2D, $F_i\big(S(u, v)\big)$, to enable simple bleb tracking and timeseries analysis.

382

383 To track blebs in 2D we computed the bounding box of individual bleb instances in every timepoint after
384 appropriate image padding to account for spherical periodicity (Fig. 4b left). In $S(u, v)$ bleb dynamics can
385 readily be followed by an established 2D multi-object bounding box tracker[77] with mean curvature optical
386 flow-guided bipartite matching (Fig. 4b middle, Methods). The bijective mapping allows us to map the
387 resulting trajectories from (u, v) coordinates (individually colored) to (x, y, z) coordinates to generate bleb
388 tracks in Cartesian 3D (Fig. 4b right, Suppl. Video 7). Due to the fast temporal acquisition rate, only a northern
389 portion of the cell can be maintained in-focus. Again, u-Unwrap3D's bijectivity between 3D and 2D
390 representation enabled us to easily map a manually annotated out-of-focus subvolume onto $S(x, y, z)$ and
391 into $S(u, v)$ to retain for analysis only the bleb tracks that remain within the in-focus surface regions (Fig. 4c).
392 The distribution of individual bleb track lifetimes showed a peak at 14s and a long tail up to 240s, suggestive
393 of a mixture of short- and long-lived blebs (Fig. 4d). The mean bleb lifetime of 27s corresponded well with a
394 30s periodicity given by the first peak of the temporal autocorrelation of mean curvature $H\big(S(x, y, z, t)\big)$ in
395 Cartesian 3D (Fig. 4e). This validates at the population level the accuracy of single bleb tracking after
396 projection and segmentation of mean surface curvature in 2D.

397 The temporal autocorrelation curves of mean curvature and Septin intensity showed a high level of similarity,
398 suggesting co-fluctuation of the two surface signals. Indeed, we had previously shown that surface regions
399 of high Septin intensity with negative surface curvature for at least 30s display a correlation between negative
400 curvature value and Septin intensity[38]. Whilst the majority of Septin pulses endured only one cycle of bleb
401 formation and retraction, *de novo* formation of stable Septin structures appeared to be driven by several
402 Septin pulses occurring in short succession. We thus hypothesized that these were formed by iterative bleb-
403 driven curvature generation events resulting in local levels of Septin oligomers surpassing a threshold
404 necessary for inter-oligomer polymerization and enabling stabilization through formation of higher-order
405 structures[38]. The ability to spatiotemporally track individual blebs enabled us now to quantitatively test this
406 model. For the duration of each tracked bleb, we sampled within the 2D bounding box distortion-corrected
407 timeseries of bleb surface area, on-/off- bleb surface mean curvature and Septin intensity (Methods). We
408 used the 2D $S(u, v)$ bounding box to define a bleb's spatial area-of-influence and its surface area. The
409 Cartesian 3D $S(x, y, z)$ box area was taken as the bleb's 3D surface area in each tracked frame. Within the
410 2D bleb bounding box, 'on-bleb' is the largest spatially contiguous region of high mean curvature. The
411 remainder area is 'off-bleb'. A single curvature threshold was computed by 3-class Otsu thresholding over all
412 $H\big(S(u, v, t)\big)$ to define the regions of low/high mean curvature in $S(u, v, t)$. Using the extracted timeseries we
413 reconstructed the temporal profile of bleb area, on-/off- mean curvature and Septin intensity of a single mean
414 bleb event in a window of 35s centered on the timepoint of maximum bleb area averaged over 545 single
415 bleb events from 480 bleb tracks. We sample ± 17.5s before and after the timepoint of maximum bleb area
416 to exceed the 30s periodicity inferred from temporal autocorrelation by 5s and capture the full dynamics. We
417 then broke the mean timeseries into four distinct temporal phases of bleb-mediated Septin recruitment, each
418 ≈ 5s long (Fig.4f labels A-D). In phase A, the bleb begins to expand, increasing its surface area, accompanied
419 by a sharp increase in on-bleb H and a decrease in Septin intensity as the plasma membrane detaches from
420 the actin cortex. The expansion also reduces off-bleb H, causing a decrease in Septin intensity off-bleb,
421 presumably due to disrupting Septin structures. In phase B, the bleb reaches maximum size and then begins
422 to retract with decreasing surface area. Interestingly, unlike mean curvature, which begins to decrease before
423 the maximum bleb area, the change in area is symmetrical, occurring ± 2.5s relative to the time of maximum
424 bleb area. Coincident with the bleb increasing to a maximum area, off-bleb H decreases to a minimum and

Septin intensity both on/off bleb stabilizes at a minimum. As the bleb retracts and the actin cytoskeleton reassembles, off-bleb H increases and Septin intensity begins to increase both on/off bleb. In phase C (+2.5s to +8.0s after peak bleb area), the bleb area continues to decrease but at a slower rate. Unexpectedly, the off-bleb negative curvature H plateaus at a value lower than its starting value (before phase A) instead of continuing to increase. Concurrently, Septin intensity undergoes the greatest rate of increase on both on-bleb and off-bleb surfaces such that at the end of phase C, the Septin intensity is at the levels before phase A (c.f. -15s to -10s). In phase D (+8.0s to +14.0s), bleb area and on/off-bleb H recover to pre-expansion levels while Septin intensity continues to increase before plateauing on both on/off-bleb surfaces. Beyond phase D, the next bleb cycle begins, with similar temporal characteristics to phase A. Altogether these results indicate that blebs generate optimal curvature dynamics during retraction to recruit Septin polymers to the surface regions around blebs. Moreover, the data support our model of Septins being recruited to negative curvature patches in a cyclic fashion, where each bleb formation-retraction drives the local accumulation of a few more oligomers until a threshold concentration is reached to trigger inter-oligomer polymerization of a stable Septin assembly. Notably, none of these observations could have been made without u-Unwrap3D.

Ruffles are driven by locally enriched filamentous actin and migrate actively on the cell surface

We also applied the capacity of u-Unwrap3D to examine putative relations between the dynamics of membrane ruffles, filamentous actin and surface actin retrograde flow. Membrane ruffles are thin, rapidly-moving, actin-rich protrusions[78,79]. They are thought to play a role in cell migration, and it has been proposed that ruffles arise as a consequence of inefficient adhesion in cellular lamellipodia[80]. Yet, it is unclear whether they have a specific function in migration or elsewhere, and the precise molecular and mechanical mechanisms of ruffle formation remain poorly understood. Although membrane ruffles have been the showcase for many of the recent advances in volumetric light sheet microscopy, there are no tools for quantitative assessment of membrane ruffling[78,81]. The thin, lamellar appearance make ruffles extremely challenging to segment in 3D live-cell images[46]. Moreover, ruffles are transient and they exhibit significant heterogeneity in nature and distribution across the cell surface. These characteristics make ruffles difficult if not impossible to directly track in Cartesian 3D.

There are many unanswered questions about cell membrane ruffles. Here, we focus on defining a membrane ruffle by a set of objective criteria and then on measuring their speed. We acquired 3D volumes of SU.86.86 pancreatic ductal adenocarcinoma cells plated on fibronectin-coated cover glasses and co-expressing Tractin-mEmerald and myristoylated CyOFP1. Tractin is a marker for actin[82], while myristoylated CyOFP1 served as a diffuse cell membrane marker. We acquired images every 2.27s for 30 timepoints, and used u-Unwrap3D to map the Cartesian 3D $S(x, y, z)$ surface of every timepoint into topographic 3D, $S(d, u, v)$ and into 2D, $S(u, v)$ for tracking (Fig. 5a, Suppl. Video 8). The cortical shape change was relatively small but ruffles travel on the surface. Like for bleb tracking we constructed a common static topographic coordinate space (d, u, v) for all time points. Here, we used the reference surface, $S_{\text{ref}}(x, y, z, t = 0)$ for this purpose (Extended Fig.5a). We then sought to track the spatial location of ruffles as individual 'ridge' objects. To do so, we projected $S(d, u, v)$ onto the 2D plane, i.e. the (u, v) coordinate was the same as setting $d = 0$, ($d = 0, u, v$) instead of applying topographic cMCF. We computed the local enrichment of the actin signal as the ratio of Tractin-mEmerald to CyOFP1 intensity. Kymograph visualization of $F_i(S(u, v, t))$ for mean curvature, H and Tractin-mEmerald/CyOFP1 (TC) showed strong co-fluctuation of curvature and local actin intensity. It also highlighted the transient, 'ripple-like' nature of ruffles and their merging and dissipation with a delay between successive ruffles of $\approx$20s (Fig. 5b). To avoid complex image processing operations such as merging and splitting we applied optical flow-based region-of-interest (ROI) tracking[83,84] to the TC intensity to track simultaneously the protrusive membrane ruffling and retrograde surface actin flow (Fig. 5c, left). The (u, v) image grid size was 1025 x 512 pixels (not 1024 x 512 as ruffles required active contour cMCF to be used to generate $S(d, u, v)$, see Methods) and the average dimensions of the tracked (u, v) ROI size was 23 x 23 pixels (approximately 0.06 x 0.11 μm pixel size in physical space). The resulting 2D ROI tracks exhibit unidirectional motion towards the cell center as shown by coloring directionality and remapping of (u, v) ROI tracks to polar (r, ϕ) (Fig. 5c, middle, Extended Fig. 5b) and Cartesian 3D (x, y, z) coordinates (Fig. 5c, right) (Suppl. Video 8). The optical flow tracks measure the geodesic cellular surface speed. To measure specifically the component corresponding to mean lateral ruffle travel speed we must project the 3D optical flow velocities along the same plane as the flat cell bottom. The volumetric imaging of cells is acquired on a cover glass tilted at $\approx$45^0 (Extended Fig. 5c, left). Direct 3D plane-fitting to $S(x, y, z)$ to determine a precise angle is sensitive to outlier points and shapes deviating from an elongated ellipsoid. Thanks to the $S(u, v)$

480 representation of u-Unwrap3D mapping the curved proximal cell surface and the planar cell bottom to the
481 upper and lower half of the unwrapped 2D image respectively, we could readily annotate in 2D (u, v) the cell
482 bottom and fit a 3D plane to only this surface patch in (x, y, z) (Extended Fig. 5c, middle, right). Similarly, we
483 could gate ROI tracks in (u, v) and compute the lateral speed only for those associated the lamellipodia and
484 lamella surface. Doing so we found two populations in the speed histogram (Fig. 5d). Visualizing the speed
485 on $S(x, y, z)$, the faster population corresponds to higher curvature ruffles with speeds ranging from 2-10
486 µm/min, and an average speed of 4.2 µm/min (Suppl. Video 8). This is at least two times faster than the
487 slower population surface retrograde actin flow ranging from 0-1 µm/min, which are consistent with the flow
488 speeds we used to measure by 2D fluorescent speckle microscopy in the lamella of epithelial cells[85]. This
489 result suggests ruffles are actively produced and transported across the cell surface. To assess the
490 synchronicity of actin and ruffles we extracted distortion-corrected timeseries of TC and mean curvature, H
491 by sampling and averaging the respective values within a spatial window of 23 x 23 centered around the
492 (u, v) coordinates of all ROI tracks on the lamellipodia and lamella surface. Averaging the temporal cross-
493 correlation curves (mean±95% confidence interval) of individual ROI tracks we find a significant positive
494 instantaneous (lag=0) correlation of 0.2 (Fig. 5e, left). Plotting the instantaneous (lag=0) correlation values
495 of individual ROI tracks as a function of the mean H value of the same ROI track and visualizing the
496 instantaneous correlation on $S(x, y, z)$, we found that ruffles with higher positive surface curvature are more
497 temporally correlated with TC intensity (Fig. 5e, middle, right). Altogether our results show that ruffles are
498 highly dynamic, transient protrusions that actively migrate on the cell surface and driven by locally enriched
499 filamentous actin. Our u-Unwrap3D framework provides now the platform for systematic investigation of the
500 mechanisms that drive and regulate these dynamics.

501

Discussion

503 Analyzing the spatiotemporal organization of molecular distributions and signaling activities on cell surfaces
504 in 3D has been limited by the lack of methods to represent, track and process these dynamics. Here we
505 introduce a surface-guided computing framework, referred to as u-Unwrap3D, to bijectively map a genus-X
506 Cartesian 3D surface to equivalent surface and volume representations that are optimally suited for a distinct
507 analytical task. The mappings rely on two critical insights: i) the engineered surface deformation of $S(x, y, z)$
508 to generate a genus-0 $S_{\mathrm{ref}}(x, y, z)$ for which a 3D spherical parameterization exists; ii) a novel, efficient
509 algorithm to relax geometric distortion on the 3D sphere in a bijective and tunable manner. Insight i) is
510 fundamental to allowing $S_{\mathrm{ref}}(x, y, z)$ to serve as a representative proxy that captures all the salient surface
511 features of the genus-X $S(x, y, z)$ surface and insight ii) to preserving this property when $S_{\mathrm{ref}}(x, y, z)$ is
512 unwrapped to the 2D plane. Using diverse cell examples we validated that u-Unwrap3D could be widely
513 applied (>90% of cases) in a manner robust to the input surface mesh quality and that it accurately captures
514 the cell geometry to transfer salient surface features, morphological or molecular, between all representations.
515 We note that this 90% is an underestimation of the applicability. The validation dataset was assembled to be
516 deliberately heterogeneous and was not segmented with the downstream aim of surface mapping. In practice,
517 segmentation algorithms continue to improve and postprocessing techniques can be used to create improved
518 surface meshes. Moreover we can leverage surface meshing algorithms that are more sophisticated than
519 marching cubes, such as dual contouring[86] and shrink-wrapping[87], to guarantee watertight mesh creation.
520 For timelapse acquisitions, as we showed in Fig. 4,5, the situation is even simpler. Only one timepoint or
521 average surface is needed to generate a single common $\bar{S}_{\mathrm{ref}}(x, y, z)$ to unwrap all timepoints. u-Unwrap3D
522 puts in place a generic platform for the spatiotemporal processing of unconstrained cell geometries.

523 u-Unwrap3D is applicable for arbitrary genus-X 3D surfaces, $S(x, y, z)$ wherever a genus-0 reference surface,
524 $S_{\mathrm{ref}}(x, y, z)$ can be found from conformalized curvature flow (cMCF). It works best when $S_{\mathrm{ref}}(x, y, z)$ is close
525 to the $S(x, y, z)$. In our current implementation, the generation of $S_{\mathrm{ref}}(x, y, z)$ may be suboptimal or fail when
526 either small handles in the mesh pinch together, causing early termination of the cMCF, or if the binary
527 voxelization and morphological hole closing fails to infill large handles and holes in the cMCF $S_{\mathrm{ref}}(x, y, z)$.
528 Whilst we can increase the range of morphological hole closing in the latter case, we restrict dilation to a
529 maximum 3-5 voxels to minimise smoothing out protrusion features in $S_{\mathrm{ref}}(x, y, z)$. Mesh surgery methods
530 have been developed in computer graphics to make non-watertight 3D meshes watertight[88,89], and fix
531 imperfections like holes and handles to reduce genus[90,91] to generate higher quality 3D meshes. These have
532 yet to be applied and fully tested for complex cell surfaces. Future development will investigate how to apply
533 such procedures to allow u-Unwrap3D to be applied to input meshes of any quality. In our data we have paid
534 attention during the acquisition to generating sufficient foreground-to-background contrast for reliable surface
535 segmentation, thus minimizing mesh defects.

The concept of geometrical reduction of 3D into 2D geometry through the choice of an optimal coordinate transformation has long existed in mathematics and physics to simplify mathematical manipulation and plotting. For example, parametric coordinates describe the sphere, the cylinder, Mobius strip and helicoids amongst others[92]. In computer graphics this is realised in the common practice of mapping between surfaces through simpler intermediaries: for example, the texture mapping of arbitrary surfaces by optimal surface cutting and mapping of the cuts into individual 2D shapes[57] and (u, v) surface parameterization by cutting and gluing individually mapped 2D planar patches[93] or mapping to canonical shapes such as the triangle[94], plane[39] or polyhedra[95] to minimize distortion. u-Unwrap3D draws inspiration from this thinking. Through the availability and development of rationalized multiple 3D-to-2D representations, u-Unwrap3D projects analyses that would otherwise require specialised mathematical operations into a sequence of simpler, computationally tractable procedures for 3D mesh processing, image processing and machine learning with specific consideration for single cell biology. Unlike computer graphics benchmarks, surface protrusions are irregular, high curvature and dense. First, we specifically chose representations that map the whole cell surface with well-behaved topologies such as the sphere and plane and designed a relaxation scheme to guarantee interpolation between the minimal conformal and area distortion. This bypasses the numerical instabilities of stitching multiple surface maps and only quasi-conformal mappings for the majority of literature methods. Second, although $S_{\mathrm{ref}}(x, y, z)$ was conceived as a mathematical trick to enable spherical parameterization, because it is derived explicitly from $S(x, y, z)$ and not as a canonical shape, it serves biologically to decompose $S(x, y, z)$ as the 'sum' of a smooth cell cortex and surface protrusions. Our results suggest the introduced $S_{\mathrm{ref}}(x, y, z)$ and $S_{\mathrm{topo}}(x, y, z)$ could open up new opportunities to model and quantify the interplay of dynamic membrane morphology and associated signals with more volumetric nuclear/cytoplasmic signalling. Lastly, we designed mappings to underlying representations, including Cartesian 3D, 3D sphere and 2D plane that are standard inputs in computer vision and machine learning.

With u-Unwrap3D standard computer vision, machine learning methods become directly applicable to computing tasks on rugged complex surfaces. More recently, research into combining different geometric representations are state-of-the-art in addressing complex computational problems such as multiple 2D image views to inform 3D mesh reconstruction[96,97], or 3D mesh vertex coordinates with 2D unwrapped images for feature extraction[98,99]. u-Unwrap3D is fully complementary to these research developments - unifying the different representations into a single surface-guided computing framework for downstream analysis. The u-Unwrap3D framework is made available as a Python library. The resources and validation provided by this work will aid the cell biology community to generate testable hypotheses of the spatiotemporal organization and regulation of subcellular geometry and molecular activity.

Acknowledgments

Author Contributions

FYZ conceived and developed u-Unwrap3D and conducted the analyses. MD generated the uShape3D protrusion instance segmentations and surfaces used in validation. AW acquired timelapse of the blebbing MV3 cell. GMG, BJC and BC acquired timelapse of the ruffling SU.86.86 cell. FYZ and GD wrote the manuscript with input from all authors. GD provided supervision and obtained funding.

Competing Interests

The authors declare no competing interests.

Data Availability

All data presented herein are available from the corresponding author upon request.

Code Availability

u-Unwrap3D is open source, developed as a Python library and is available at: https://github.com/DanuserLab/u-unwrap3D.

590

591

Methods

u-Unwrap3D framework

Following we describe the algorithms underpinning each step of u-Unwrap3D depicted in Fig.1.

Step 1: $S(x, y, z)$ to $S_{ref}(x, y, z)$

Conformalized mean curvature flow (cMCF). cMCF[49] modifies the mean curvature flow (MCF) to avoid the formation of pinches and collapsed vertices that compromise bijectivity and cause early flow termination for watertight meshes with high curvature features. We find cMCF also reduces the size of holes and handles in $S(x, y, z)$. MCF evolves Φ_t, the mesh at time t according to $\frac{\partial \Phi_t}{\partial t} = \Delta \Phi_t$, where Δ is the Laplace-Beltrami operator induced by the metric g_t and $\Phi_t(p) = \sum_{i=1}^{N} v_i(t) B_i(p)$ is the discrete mesh parameterization with N vertex positions $v_i(t) = \{v_1(t), \ldots, v_N(t)\} \subset \mathbb{R}^3$, each $v_i(t)$ a 3D $(x_i(t), y_i(t), z_i(t))$ coordinate tuple and $\{B_1, \ldots, B_N\}$ the local function basis, which for a triangle mesh is the linear hat basis spanned by the edge vectors. Galerkin's method[100] is used to find a weak, least-squares solution to the MCF equation within the span of $\{B_i\}$, by solving $\int_S \left(\frac{\partial \Phi_t}{\partial t} \cdot B_i \right) d\mu_t = \int_S (\Delta_t \Phi_t \cdot B_i) d\mu_t$, $\forall\ 1 \le i \le N$. S is the surface spanned by $\{B_i\}$ and $d\mu_t$ the volume form. The equation is solved to obtain the vertex position, $v(t + \delta t)$ at the next iteration, $t + \delta t$ using backwards Euler integration; $\frac{\partial \Phi_t}{\partial t} \approx (\Phi_{t+1} - \Phi_t)/\delta_t$, $\Delta \Phi_t \approx \Delta \Phi_{t+1}$ and noting the Laplace-Beltrami is the divergence of the gradient, $\Delta = \nabla \cdot \nabla$ with respect to the local mesh metric g_t to get $\int_S (\sum_{i=1}^{N} v_i(t + \delta t) B_i \cdot B_j - \sum_{i=1}^{N} v_i(t) B_i \cdot B_j) d\mu_t = \delta_t \int_S \sum_{i=1}^{N} v_i(t + \delta t) g_t(\nabla_t B_i, \nabla_t B_j) d\mu_t$. The integrals, $M_{ij}^t = \int_S B_i \cdot B_j\, d\mu_t$ and $L_{ij}^t = \int_S g_t(\nabla_t B_i, \nabla_t B_j) d\mu_t$ are called the mass ($\mathbf{M}_t$) and Laplacian ($\mathbf{L}_t$) matrices at time, t. Substituting this notation and rearranging, the linear algebra MCF equation is

$$MCF := (\mathbf{M}_t - \delta_t \mathbf{L}_t) v(t + \delta t) = \mathbf{M}_t v(t)$$

$v(t + \delta t)$ is then computed from $v(t)$ by direct matrix inversion. cMCF modifies the MCF equation above by using the Laplacian matrix at time $t = 0$, $\mathbf{L}_0$ for all timepoints. The Laplacian matrix is a measure of stiffness between local mesh faces, see active contour cMCF below. Using $\mathbf{L}_0$ for all timepoints instead of recomputing implicitly constrains mesh faces to retain the same aspect ratio and this conformalizes the flow.

$$cMCF := (\mathbf{M}_t - \delta_t \mathbf{L}_0) v(t + \delta t) = \mathbf{M}_t v(t)$$

We use the *libigl* library[101] with the cotangent Laplacian and barycentric mass matrix as the default implementations of $\mathbf{M}_t$, $\mathbf{L}_t$ respectively. We improve the numerics of solving cMCF by normalization of the surface area and recentering of vertex coordinates at the origin after each iteration as recommended in Alec Jacobson's blog post (https://www.alecjacobson.com/weblog/?tag=mean-curvature-flow). u-Unwrap3D implements for optional usage the robust Laplacian of Sharp et al.[102] instead of the cotangent Laplacian, which can improve numerical stability.

623

Automatic stopping criterion for cMCF. The ideal reference surface $S_{ref}(x, y, z)$ for topographic representation is the cortical cell shape without protrusions. We find that this corresponds to finding the 'elbow point' in the mean absolute Gaussian curvature (Fig. 1a) and not the convergence limit of cMCF which is the sphere[49]. The difference in the mean absolute Gaussian curvature over vertices, $\Delta \overline{|K_t|} = \overline{|K_t|} - \overline{|K_{t-1}|}$ between successive iterations $t - 1$ and t is used as an automatic stopping criterion for cMCF, $stop_t = \max(t_{min}, t_K)$. t_{min} is a user-specified minimum iteration number and t_K is the first iteration for which $\Delta \overline{|K_t|}$ exceeds a user-specified threshold, $\Delta \overline{|K_t|} > \Delta_{thresh}$. We compute the discrete Gaussian curvature, K_{v_i} at a vertex v_i, given by the vertex's angular deficit[103], $K_{v_i} = 2\pi - \sum_{j \in N(i)} \theta_{ij}$ where $N(i)$ are the triangles incident on v_i and θ_{ij} is the angle at vertex i in triangle j.

633

634 *Mesh voxelization and remeshing*. Mesh voxelization converts a surface mesh $S(x, y, z)$ to a binary volume
635 image β where individual voxels are either 1 if they are interior to the surface or 0 if exterior. To do this we
636 create a $X \times Y \times Z$ voxel volume image larger than the surface with voxels initialised to 0. We then set the
637 intensities of all voxels indexed by the mesh (x, y, z) barycenters to 1, i.e. $B(x, y, z) = 1$. To ensure a closed
638 binary volume with all interior voxel intensities = 1, $S(x, y, z)$ was iteratively subdivided by replacing each
639 triangle face by four new faces formed from adding new vertices at the midpoint of every edge until the mean
640 triangle edge length is < 1 voxel. We use the barycenter coordinates of the final mesh $S_{final}(x, y, z)$ to set the
641 binary voxel values. In case of small holes in $S(x, y, z)$ that would prevent a closed binary volume by binary
642 filling only, β was first dilated with a ball kernel, then binary infilled, and lastly binary eroded with a ball kernel.
643 The postprocessed B was meshed using marching cubes[104] followed by construction of an approximated
644 centroidal voronoi diagram (ACVD)[105] to produce a lower genus remeshing of $S(x, y, z)$ with approximately
645 equilateral triangle faces. If the input $S(x, y, z)$ is a smooth shape with only small holes or handles such as
646 the cMCF intermediary $S_{ref}(x, y, z)$ the proposed voxelization and remesh yields a genus-0 mesh.

647

648 *Step 2:* $S_{ref}(x, y, z)$ to $S_Q^2(x, y, z)$

649 *Quasi-conformal spherical parametrization of genus-0 closed surfaces*. For genus-0 closed surfaces the
650 uniformization theorem[52] guarantees the existence of a conformal map onto the unit sphere, $\mathbb{S}^2$. For a closed
651 orientable surface such as $S_{ref}(x, y, z)$ we can compute the genus, g from the Euler characteristic, $\chi = 2 -$
652 $2g = \#V - \#E + \#F$. If $g = 0$ we applied the method of Choi et al.[39,51] which uses the theory of quasi-
653 conformal composition to ensure a bijective spherical parametrization with bounded conformal error (i.e.
654 quasi-conformal). In practice, we found conformal errors = 0 (Extended Fig. 3).

655

656 *Step 3:* $S_Q^2(x, y, z)$ to $S_\Omega^2(x, y, z)$

657 *Equiareal spherical parameterization by mesh relaxation*. We iteratively advect the vertex coordinates of
658 $S_Q^2(x, y, z)$, whilst preserving face connectivity and the spherical shape to minimise the per face area distortion
659 factor, λ. The magnitude and direction to advect each vertex, the vector field $\vec{V}$ was found as the solution to
660 the linear heat equation[27], $\frac{d\lambda}{dt} = -\Delta\lambda$, where Δ denotes the Laplacian. This is because an infinitesimal change
661 in λ in the direction of $\vec{V}$ is the Lie-derivative on 2-forms, $\mathcal{L}_{\vec{V}}\lambda = -\nabla \cdot (\lambda\vec{V})$ such that we can set $\Delta\lambda = -\nabla \cdot$
662 $(\lambda\vec{V})$. As the Laplacian is the divergence of the gradient, $\Delta\lambda = \nabla \cdot (\nabla\lambda)$ we have $\vec{V} = -\frac{\nabla\lambda}{\lambda} = -\nabla\log\lambda$. To ensure
663 vertices are displaced geodesically on the surface of a sphere according to $\vec{V}$, instead of unwrapping the
664 sphere to the 2D (u, v) plane as in Lee et al[27], we developed a direct 3D advection scheme that displaces
665 vertices in small constant step sizes ϵ using active contour cMCF (see below), and reprojecting to the sphere.
666 λ and $\vec{V}$ are recalculated for the new vertex positions and advection is repeated until an equiareal
667 parameterization was achieved or the maximum number of iterations was reached. Details of our advection
668 scheme is given algorithmically.

Input: Conformal spherical parameterization mesh, $S_Q^2(x, y, z)$ with vertices, v_{sphere} and faces, f_{sphere}, matching genus-0 mesh, $S_{ref}(x, y, z)$ with vertices, v_{ref} and faces, f_{ref} with identical number of vertices, $|v_{sphere}| = |v_{ref}|$, and faces, $f_{sphere} = f_{ref}$; vertex step size, ϵ; total number of iterations, T; mesh stiffness factor, δ (also known as the time step, δ in cMCF)

For iterations $t = 1, 2, \ldots, T \ldots$ do :

$a_{ref}^{f_i} \leftarrow a_{ref}^{f_i} / \Sigma_i a_{ref}^{f_i}$ (normalised area per face f_i of S_{ref})

$a_{sphere}^{f_i}(t) \leftarrow a_{sphere}^{f_i}(t) / \Sigma_{f_i} a_{sphere}^{f_i}(t)$ (normalised area per face f_i of S_Q^2)

$\lambda^{f_i}(t) \leftarrow a_{ref}^{f_i} / a_{sphere}^{f_i}(t)$ (area distortion factor per face f_i of S_Q^2)

$\vec{V}^{f_i}(t) = -\nabla\log\lambda^{f_i}(t)$ (compute ∇ using the mesh grad operator[106], per face f_i)

$\vec{V}^{f_i}(t) \leftarrow \left(\frac{\bar{l}}{median(\|\vec{V}^{f_i}(t)\|)}\right)\vec{V}^{f_i}(t)$ (normalize the displacement vector with respect to the average triangle edge length, $\bar{l}$)

$\lambda^{v_i}(t) \leftarrow average(\lambda^{f_i}(t))$,   (average the per face f_i vector field and area distortion

$\vec{V}^{v_i}(t) \leftarrow average\left(\vec{V}^{f_i}(t)\right)$ factor onto vertices, v_i)

$$\vec{V}^{v_i}_{tangent}(t) \leftarrow \vec{V}^{f_i}(t) - \left(\vec{V}^{f_i}(t) \cdot \vec{N}^{v_i}(t)\right)\vec{N}^{v_i}(t)$$

(compute the surface tangential component of $\vec{V}^{v_i}(t)$ using per vertex normal, $\vec{N}^{v_i}(t)$)

$$v_{sphere}(t+1) = \left(\mathbf{M}(t) - \delta_t\mathbf{L}(t)\right)^{-1}\mathbf{M}(t)\left(v_{sphere}(t) + \epsilon\vec{V}_{tangent}(t)\right)$$

(perform active contour cMCF to advect v_{sphere} in direction of $\vec{V}^{v_i}_{tangent}(t)$ and compute the new v_{sphere})

$$v_{sphere}(t+1) \leftarrow v_{sphere}(t+1)/\|v_{sphere}(t+1)\|$$

(Ensure v_{sphere} lies on a sphere by centroid distance normalization)

669 This mesh relaxation bijectively diffuses the area distortion scalar factor on the sphere surface in a stable
670 manner until a triangle face collapses, that is when an interior angle = 0. The extent of area relaxation is
671 determined by the input mesh quality. We find that if $S_{\mathrm{ref}}(x,y,z)$ is regular, with near-equilateral faces and
672 has a minimal number of vertices (> $40k$ for a pixel resolution of $0.104\mu m$), $S^2_Q(x,y,z)$ could be stably relaxed
673 to an equiareal spherical parameterization, $S^2_\Omega(x,y,z)$, without triangle collapse.

674

675 *Step 4:* $S^2_\Omega(x,y,z)$ *to* $S(u,v)$

676 *Automatic determination of unwrapping axis using weighted principal components analysis (PCA).* Mapping
677 surface features of interest with minimal geometrical distortion into $S(u,v)$ is equivalent to finding an optimal
678 north-south unwrapping axis for $S^2_\Omega(x,y,z)$. This optimization is solved by weighted PCA. Let $v_i = (x_i, y_i, z_i)$
679 denote the coordinate of vertex i on $S^2_\Omega(x,y,z)$ and w_i the vertex weight, a score of the importance of
680 mapping this vertex with minimum geometrical distortion. The 3x3 weighted covariance matrix, $\mathbf{A} =$
681 $(\boldsymbol{w}^T\boldsymbol{v})(\boldsymbol{w}^T\boldsymbol{v})^T$ over all vertices captures the spread of the weight over the sphere. Eigendecomposition
682 applied to the symmetric matrix $\mathbf{A}$ finds the principal orthogonal directions of variance given by eigenvalues
683 $\boldsymbol{\lambda} = [\lambda_1, \lambda_2, \lambda_3]$, $\lambda_1 \geq \lambda_2 \geq \lambda_3$ and eigenvectors $\mathbf{e} = [\mathbf{e_1}, \mathbf{e_2}, \mathbf{e_3}]$. The eigenvalue captures the concentration of
684 the weight w in the direction of the corresponding eigenvector. The optimal north-south unwrapping axis is
685 the smallest eigenvector, $\mathbf{e_3}$. To unwrap with respect to $\mathbf{e_3}$ we rotate the vertex coordinates v_i so that $\mathbf{e_3}$ is
686 the new *z*-axis. As the eigenvector matrix $\mathbf{e}$ is orthonormal and thus a 3D rotation matrix, $\mathbf{e}$ is the rotation
687 matrix $\mathbf{R}$ that maps the *x*-axis, $\begin{pmatrix}1\\0\\0\end{pmatrix} \mapsto \mathbf{e_1}$, *y*-axis, $\begin{pmatrix}0\\1\\0\end{pmatrix} \mapsto \mathbf{e_2}$, *z*-axis, $\begin{pmatrix}0\\0\\1\end{pmatrix} \mapsto \mathbf{e_3}$. For a 'pure' or proper rotation
688 matrix without reflection the determinant of $\mathbf{R}$ must be +1, $\det(\mathbf{R}) = +1$. We derive a proper rotation, $\mathbf{R}' =$
689 $[\mathbf{e_1}', \mathbf{e_2}', \mathbf{e_3}']$ from $\mathbf{R}$ by flipping the sign of $\mathbf{e_1}$, $\mathbf{e_2}$ to have positive x- and y- components respectively; $\mathbf{e}'_1 \leftarrow$
690 $sgn(v_{1x})\mathbf{e_1}$, $\mathbf{e}'_2 \leftarrow sgn(v_{2y})\mathbf{e_2}$ (sgn is the sign function) and constructing $\mathbf{e}'_3 = \mathbf{e}'_1 \times \mathbf{e}'_2$ as the cross product of
691 $\mathbf{e}'_1$ and $\mathbf{e}'_2$. The matrix inverse of $\mathbf{R}'$ (also the matrix transpose, $\mathbf{R'}^\mathbf{T}$) is the desired rotation of $S^2_\Omega(x,y,z)$ such
692 that $\mathbf{e}'_3$ is the new *z*-axis. $\mathbf{R'}^\mathbf{T}$ maps the eigenvectors, $\mathbf{e}'_1 \mapsto \begin{pmatrix}1\\0\\0\end{pmatrix}$, $\mathbf{e}'_2 \mapsto \begin{pmatrix}0\\1\\0\end{pmatrix}$, $\mathbf{e}'_3 \mapsto \begin{pmatrix}0\\0\\1\end{pmatrix}$.

693

694 *UV-mapping the unit sphere.* We construct an equidistant UV unwrap of the unit sphere where u, the column
695 coordinate equidistantly samples the circumference of the sphere, a total length 2π and v, the row coordinate
696 equidistantly samples the arc from north to south pole, a total length π. This specifies a $N \times 2N$ pixel UV
697 image with N as a user-defined size. By default $N = 256$ pixels. The UV mapping is constructed by pullback.
698 Let $u = \theta$ be the azimuthal and $v = \varphi$ be the inclination angles of the sphere and setup the $N \times 2N$ grid of v
699 vs u over the parameter space $[-\pi, 0] \times [-\pi, \pi]$. Convert the spherical coordinates, $(1, \theta, \varphi)$ to cartesian
700 coordinates, $(x,y,z) = (\sin\theta\cos\varphi, \sin\theta\sin\varphi, \cos\theta)$. Each (x,y,z) coordinate is matched by nearest
701 distance to a triangle face, ABC of $S^2_\Omega(x,y,z)$ to compute barycentric coordinates giving (x,y,z) as a convex
702 combination of the vertices A, B, C; $\mu_A A + \mu_B B + \mu_C C$, $\mu_A, \mu_B, \mu_C \geq 0$ and $\mu_A + \mu_B + \mu_C = 1$. By bijectivity of
703 $S_{\mathrm{ref}}(x,y,z)$ and $S^2_\Omega(x,y,z)$, we set A, B, C to the respective $S_{\mathrm{ref}}(x,y,z)$ vertex coordinates to produce the
704 respective uv- coordinate mapping, $(u,v) \leftrightarrow \mu_A A + \mu_B B + \mu_C C$. Note, setting A, B, C to be the vertex
705 coordinates of any mesh bijective to $S^2_\Omega(x,y,z)$ e.g. $S(x,y,z)$ in the direct unwrapping case produces similarly
706 the corresponding uv- coordinate mapping for that mesh. The weights μ_A, μ_B, μ_C is also used to map any other
707 vertex associated quantities, $I_i\left(S^2_\Omega(x,y,z)\right)$ such as curvature to $I_i(S(u,v))$, with vector-valued vertices A, B, C
708 replaced now by scalar values. The construction of the UV map as described above replicates the first and
709 last column of the resulting UV image. For applications such as texture mapping and active contour cMCF
710 (see analysis of ruffles in Datasets section) where the UV image grid needs to be converted to a triangular
711 mesh and the image boundaries 'stitched' together we instead use a $N \times 2N + 1$ pixel UV image.

712

713 *Step 5:* $V(x, y, z)$ to $V(d, u, v)$

714 *Topographic coordinate space* (d, u, v) *construction.* UV-unwrapping establishes bijection between a 2D uv
715 plane and a 3D Cartesian surface, $(u, v) \leftrightarrow S_{\text{ref}}(x, y, z)$. We construct a topographic (d, u, v) coordinate
716 space, $V(d, u, v)$ corresponding to a volume space normal to $S_{\text{ref}}(x, y, z)$ by propagating the (u, v)
717 parameterized $S_{\text{ref}}(x, y, z)$ in Cartesian 3D at equidistant steps of α voxels, referred to as α -steps, along the
718 steepest gradient of the signed distance function, $\nabla\Phi(x, y, z)$ for a total of D steps, $d \in -D_{in}, \ldots, D_{out}$. $D =$
719 $D_{out} + D_{in}$ is the total number of α -steps outwards and inwards relative to $S_{\text{ref}}(x, y, z)$ (which is $d = 0$)
720 respectively. D_{out} is automatically determined to ensure $V(d, u, v)$ fully encapsulates $S(x, y, z)$. D_{in} is user-
721 defined for computational efficiency or automatically determined as a fraction of the maximum internal
722 distance transform. The signed distance function $\Phi(x, y, z)$ of $S_{\text{ref}}(x, y, z)$ is computed from the binary volume
723 after voxelization. We voxelize the (u, v) parameterized $S_{\text{ref}}(x, y, z)$ directly using the same procedure as for
724 meshes but employ image upscaling instead of mesh subdivision to ensure that the distance of 1 pixel in the
725 (u, v) space is < 1 voxel in Cartesian (x, y, z) space. Using active contour cMCF (see below) to propagate
726 the (u, v) parameterized $S_{\text{ref}}(x, y, z)$ for large D is slow; a 256x512 UV unwrap is 131,072 vertices. Moreover
727 for a large D_{out} as the intra-spacing of 3D (x, y, z) positions increases, numerical instabilities arise that
728 require implicit Laplacian smoothing[107] to suppress, which is also slow. Instead we use explicit Euler
729 integration for propagation; $S_{\text{ref}}(x, y, z)_{d+\alpha} = S_{\text{ref}}(x, y, z)_d + \alpha \frac{\nabla\Phi_{S_{\text{ref}(x,y,z)_d}}}{\left|\nabla\Phi_{S_{\text{ref}(x,y,z)_d}}\right|}$ at α voxels from $S_{\text{ref}}(x, y, z)_d$ and
730 $\frac{\nabla\Phi_{S_{\text{ref}(x,y,z)_d}}}{\left|\nabla\Phi_{S_{\text{ref}(x,y,z)_d}}\right|}$ is the unit gradient of $\Phi(x, y, z)$. Computationally efficient image-based filtering is then applied to
731 smooth $S_{\text{ref}}(x, y, z)_{d+\alpha}$ per iteration to maintain bijectivity and suppress instabilities. Tilinear interpolation of
732 the respective Cartesian volumetric signal intensities, $I_i(V(x, y, z))$ at the (x, y, z) coordinates indexed by
733 $V(d, u, v)$ generates the topographic 3D equivalents, $I_i(V(d, u, v))$.

734

735 *Step 6:* $I_i(V(d, u, v))$ to $S(d, u, v)$
736 *Topographic mesh* $S(d, u, v)$ *construction.* $S(x, y, z)$ was voxelized to a binary volume, $I_i(V(x, y, z))$ as above
737 and transformed to $I_i(V(d, u, v))$. Marching cubes were applied at isovalue = 0.5 to create an initial $S(d, u, v)$
738 which was remeshed with ACVD to construct the final low-genus $S(d, u, v)$ with near-equilateral triangle faces.
739 The Cartesian 3D mesh, $S_{\text{topo}}(x, y, z)$ of $S(d, u, v)$ was constructed by interpolation of the (x, y, z) coordinates
740 indexed by the corresponding (d, u, v) coordinates. To transform surface signals, $F_i(S(x, y, z))$ to
741 $F_i(S(d, u, v))$, nearest neighbors was used to match $S(x, y, z)$ and $S_{\text{topo}}(x, y, z)$.

742

743 <u>Mesh displacement by active contour cMCF</u>
744 Active contours, or 'snakes' [108], define the boundary of an image region by minimizing its contour energy, E.
745 The contour energy is the sum of an internal, E_{int} and an external energy, E_{image}, $E(v, I) = E_{image}(v, I) +$
746 $E_{int}(v)$. The internal energy is set by $E_{int} = \int \alpha|v'|^2 + \beta|v''|^2 \, ds$, where the number of ' denotes the order of
747 the spatial derivative. Here, the first term is the tension and α the elasticity of the contour. The second term
748 is the stiffness and β the rigidity of the contour. The external energy is set by $E_{image} = -\int p \, ds$, where p is
749 an attractor image for the contour. Minimizing E is equivalent to solving the Euler-Lagrange equation, $\alpha v'' -$
750 $\beta v'''' = -\nabla p$ or in matrix form, $\mathbf{A}v + \nabla p = 0$, where $\mathbf{A}$ prescribes the constant coefficients for computing the
751 second and fourth order derivatives by finite differences. Given a vertex position $v(t)$, the next position, $v(t +$
752 $1)$ is computed that minimises the residual error $\mathbf{A}v + \nabla p$ using gradient descent and backwards Euler is
753 $v(t + 1) = v(t) - (\mathbf{A}_t v(t + 1) + \nabla p)$ and the linear system is $(\mathbf{I} + \mathbf{A}_t)v(t + 1) = v(t) + \nabla p$, where $\mathbf{I}$ is the
754 identity matrix. If $\beta = 0$, $\mathbf{A}_t$ only comprises the second order coefficients associated with v'', and we have
755 $(\mathbf{I} + \alpha_t \mathbf{M}_t^{-1} \mathbf{L}_t)v(t + 1) = v(t) + \nabla p$ or equivalently, $(\mathbf{M}_t - \alpha_t \mathbf{L}_t)v(t + 1) = \mathbf{M}_t(v(t) + \nabla p)$ which is identical to
756 the cMCF equation with $\mathbf{M}_t$ the mass matrix, $\mathbf{L}_t$, the Laplacian matrix, $\alpha_t = \delta_t$ and $\mathbf{L}_t = \mathbf{L}_0$ in response to an
757 external force, ∇p. This general equation can be solved by direct matrix inversion to move surface meshes
758 diffeomorphically. We refer to this as *active contour cMCF* in this paper. To evolve mesh vertices, $v(t)$ normal
759 to the surface in equal α-steps, we set $p = \Phi$, the signed distance function, and solve $(\mathbf{M}_t - \alpha_t \mathbf{L}_t)v(t + 1) =$
760 $\mathbf{M}_t(v(t) + \alpha\nabla\Phi)$ iteratively, with $\nabla\Phi$ evaluated at $v(t)$ for each iteration. A positive α moves a cell surface
761 mesh normally outwards from the cell and a negative α moves the mesh normally into the cell.

762

763 <u>Quantification of geometric deformation errors for meshes</u>
764 Surface mappings do not conserve local geometrical measures like angles, edge lengths and face area.
765 Quantification of the distortion in these measures enables a task-specific optimization of the mapping and
766 correction of statistical measurements made on the mapped surface. There are two primary geometric
767 distortions to quantify; conformal and area distortion error (Extended Fig. 1). An isometric deformation is one
768 with no error; both conformal and area distortion errors are 0.

769

770 *Conformal error*. The conformal or quasi-conformal error, Q_i measures the extent the shape of a mesh
771 element i, e.g. a triangle face, is stretched. It is 0 if the relative distances between vertices and the angles
772 between edges are preserved after the mapping. We compute Q of mapping triangle ΔABC to ΔDEF in 3D by
773 first isometrically projecting all triangles into 2D. Let $A = (x_1, y_1, z_1)$, $B = (x_2, y_2, z_2)$, $C = (x_3, y_3, z_3)$ with
774 edge vectors, $\overrightarrow{AB} = B - A$, $\overrightarrow{AC} = C - A$, then an identical 2D triangle $\Delta A'B'C'$ is given by $A' = (0,0)$, $B' =$
775 $(|\overrightarrow{AB}|, 0)$, $C = (\overrightarrow{AB} \cdot |\overrightarrow{AB} \times \overrightarrow{AC}|)$, $A', B', C' \in \mathbb{R}^2$. Let $\mathbf{X} = \begin{bmatrix} A' & B' & C' \\ 1 & 1 & 1 \end{bmatrix}$, be the 3x3 homogeneous vertex
776 coordinates of $\Delta A'B'C'$ and $\mathbf{Y} = \begin{bmatrix} D' & E' & F' \\ 1 & 1 & 1 \end{bmatrix}$, the 3x3 homogeneous vertex coordinates of $\Delta D'E'F'$ then we
777 solve for the 3x3 matrix, $\mathbf{A}$ that maps $\mathbf{X}$ to $\mathbf{Y} = \mathbf{AX}$. $\mathbf{A}$ is affine and of the form $\begin{bmatrix} \mathbf{J} & | & \mathbf{T} \\ \mathbf{0} & | & 1 \end{bmatrix}$ where $\mathbf{J}$ is a 2x2
778 transformation matrix and $\mathbf{T}$ a translation matrix. Eigenvector decomposition of $\mathbf{J^T J}$ gives 2 eigenvalues λ_1, λ_2,
779 $\lambda_1 < \lambda_2$ and the singular values of $\mathbf{J}$, $\sigma_1 = \sqrt{\lambda_1}, \sigma_2 = \sqrt{\lambda_2}$. The ratio $\frac{\sigma_2}{\sigma_1}$ is the conformal error[62]. The global
780 conformal error, Q of deforming a surface mesh S_1 to a mesh S_2 is the area weighted average of individual
781 conformal errors Q_{f_i} of each triangle face f_i in S_1; $Q = \frac{\Sigma_{f_i \in S_1} a_{f_i} Q_{f_i}}{\Sigma_{f_i \in S_1} a_{f_i}}$ where a_{f_i} is the area of face f_i of S_1.

782

783 *Area distortion error*. The area distortion error, λ measures the extent the surface area fraction of a mesh
784 face is preserved during a surface mapping. One measure of λ is $\sigma_1 \sigma_2$, the product of the singular values of
785 $\mathbf{J}$ and the area of the distortion ellipse[63]. Here we use the surface area fraction ratio, $\lambda_{\Delta ABC} = \frac{\frac{area(\Delta ABC)}{surface\ area\ of\ S_1}}{\frac{area(\Delta DEF)}{surface\ area\ of\ S_2}}$
786 as a direct measurement of the area distortion in mapping ΔABC to ΔDEF in 3D. The global area distortion
787 error, $\lambda = \frac{1}{|f_i|} \Sigma_{f_i \in M_1} \lambda_{f_i}$ for mapping a mesh S_1 to a mesh S_2 is the mean over all individual area distortion λ_{f_i}
788 of each triangle face f_i in S_1, with $|f_i|$ the number of faces in S_1 and $\lambda_{f_i} = \frac{a_{fi}^{S_1}/\Sigma_{f_i \in S_1} a_{fi}^{S_1}}{a_{fi}^{S_2}/\Sigma_{f_i \in S_2} a_{fi}^{S_2}}$ is the area distortion of
789 face f_i. The normalization of face area by total surface area is crucial to enable the computation of λ
790 independent of scale.

791

792 <u>Quantification of geometric deformation error for UV images</u>
793 UV mapping defines a bijective relation between the 2D (u, v) rectilinear grid and a 3D surface, $S(u, v) \leftrightarrow$
794 $S = S(x(u,v), y(u,v), z(u,v))$. Differentials can be used to compute geometric quantities when the (u, v)
795 spacing is comparable to the (x, y, z) spacing. The differential area of a (u, v) pixel is $dA = \left| \frac{\partial S}{\partial u} \times \frac{\partial S}{\partial v} \right| du\ dv$,
796 where $\frac{\partial S}{\partial u} = \left(\frac{\partial S_x}{\partial u}, \frac{\partial S_y}{\partial u}, \frac{\partial S_z}{\partial u} \right)$ and $\frac{\partial S}{\partial v} = \left(\frac{\partial S_x}{\partial v}, \frac{\partial S_y}{\partial v}, \frac{\partial S_z}{\partial v} \right)$ are the image gradients of the x, y, z surface coordinates
797 in u, v directions. The topographic space construction establishes bijection of the 3D (d, u, v) volumetric grid
798 to a 3D volume, V, $(u, v, d) \leftrightarrow V = V(x(u,v,d), y(u,v,d), z(u,v,d))$. The differential volume of a (d, u, v) voxel
799 is $dV = \left| \left(\frac{\partial V}{\partial u} \times \frac{\partial V}{\partial v} \right) \cdot \frac{\partial V}{\partial d} \right| du\ dv\ dd$. The matrix $\left[\frac{\partial S}{\partial u}, \frac{\partial S}{\partial v} \right]$ is the 2x3 Jacobian matrix, $\mathbf{J}$ and the conformal error per
800 pixel is $\frac{\sigma_2}{\sigma_1}$, $\sigma_1 = \sqrt{\lambda_1}, \sigma_2 = \sqrt{\lambda_2}$ where λ_1, λ_2, $\lambda_1 < \lambda_2$ are the two eigenvalues of $\mathbf{J^T J}$. The global conformal error
801 Q of uv-mapping the surface mesh S is the differential area weighted average of individual conformal errors
802 Q_{uv} of each uv pixel; $Q = \frac{\Sigma_{uv} dA_{uv} Q_{uv}}{\Sigma_{uv} dA_{uv}}$ where dA_{uv} is the area element of the uv pixel. The area distortion error
803 per uv pixel is the ratio between the surface area fraction of a uv pixel and the corresponding surface element
804 on S, $\lambda_{uv} = \frac{\frac{dudv}{\Sigma_{uv} dudv}}{\frac{dA_{uv}}{\Sigma_{uv} A_{uv}}}$. Note $dudv = 1$ and $\Sigma_{uv} dudv =$ total number of uv pixels. The global area distortion error,
805 $\lambda = \frac{1}{|uv|} \Sigma_{uv} \lambda_{uv}$ for uv mapping a surface S is the mean over all individual area distortion λ_{uv} of each uv pixel.

806

807 <u>Stopping criteria for area distortion relaxation of $S^2_\Omega(x, y, z)$</u>
808 We used three additional stopping criteria to demonstrate intermediate area distortion relaxation between
809 fully conformal, $S^2_Q(x, y, z)$ and fully equiareal, $S^2_\Omega(x, y, z)$ parameterization. We use the same nomenclature
810 as for the above discussed geometric deformation error for meshes.

811

812 *Most isometric parametrization (MIPS) error*. The MIPS[62] error is defined $\frac{\sigma_2}{\sigma_1} + \frac{\sigma_1}{\sigma_2}$ and is minimal when $\sigma_1 =$
813 σ_2. This error is trivially minimal for a conformal spherical parametrization $\left(\frac{\sigma_1}{\sigma_2} = 1 \right)$ (Extended Fig. 3b).

814

815 *Area-preserving MIPS*. The area-preserving MIPS[63] is defined $\left(\frac{\sigma_1}{\sigma_2} + \frac{\sigma_2}{\sigma_1}\right)\left(\sigma_1\sigma_2 + \frac{1}{\sigma_1\sigma_2}\right)^{\theta}$. We use this metric
816 with $\theta = 1$, which measures the uniformity of stretch distortion over a surface. This error yields near-equiareal
817 spherical parametrization (Extended Fig. 3b).

819 *Isometric error*. We observed tradeoff of conformal error, Q and log area distortion, $\log\lambda$ on a similar
820 magnitude scale such that their summation has a unique global minima (Extended Fig. 1e). We thus define
821 an isometric error metric, $(1-\theta)\frac{\sigma_1}{\sigma_2} + (\theta)\log\lambda$ with a constant $\theta \in [0,1]$ to weight the relative importance of
822 jointly minimizing conformal and area distortion error. We use $\theta = 0.5$ in Extended Fig. 3b.

824 Assessment of geometrical difference between two meshes

826 Four metrics were used to assess the difference between two meshes S_1 and S_2 possessing different number
827 of vertices and faces; chamfer distance (CD), Wasserstein-1 distance (W_1), the difference in surface area
828 (ΔA) and the difference in volume (ΔV). CD is the mean Euclidean distance between all vertices of S_1 when
829 matched to the nearest vertex of S_2 and vice versa, $CD = \frac{1}{|S_1|}\sum_{x \in S_1}\min_{y \in S_2}\|x - y\|_2 + \frac{1}{|S_2|}\sum_{y \in S_2}\min_{x \in S_1}\|x - y\|_2$. The
830 Wasserstein-1 distance (W_1) or Earth-mover's distance (EMD) is the minimum total area weighted distance
831 of 1-to-1 matching vertices on S_1 and S_2. W_1 accounts for the area of triangle faces and is minimal if the
832 vertices of S_1 is a uniform sampling of S_2 or vice versa. Exact computation of W_1 is impractical, even for small
833 meshes. We compute W_1 using the sliced-Wasserstein, SW_1 approximation, which uses random spherical
834 projections to sum multiple 1D EMD distances[64]. Specifically we use the
835 *ot.sliced.max_sliced_wasserstein_distance* function from the Python *POT* library with 50 projections and
836 average the result from 10 evaluations to report an estimate. The difference in total surface area is $\Delta A = $
837 $A_{S_1} - A_{S_2}$ and is $\Delta A(\%) = \frac{A_{S_1} - A_{S_2}}{A_{S_2}}$ when given as a percentage. Total surface area was computed as the sum
838 of individual triangle areas. The difference in total volume is $\Delta V = V_{S_1} - V_{S_2}$ and is $\Delta V(\%) = \frac{V_{S_1} - V_{S_2}}{V_{S_2}}$ when
839 given as a percentage. The volume of a mesh was computed as the number of voxels in its binary voxelization
840 computed as described above. We used the minimal possible dilation ball kernel size to ensure a correct
841 volume computation - visual checking of binary voxelization and value at least 3x surface area. We use $S_1 = $
842 $S_{\text{topo}}(x, y, z)$ and $S_2 = S(x, y, z)$ to compute the metrics of Extended Fig. 2,3.

844 Reference surface inference for measurement of protrusion height

845 An optimal reference surface for protrusion segmentation must be a (u, v) parameterized surface i.e.
846 $S_{\text{ref}}(d_{\text{ref}} = f(u, v), u, v)$ where $f(\cdot)$ is injective such that every surface point is defined by a unique (d, u, v)-
847 tuple. We prove this by contradiction. Suppose a surface, $S(d, u, v)$ has points with the same (u, v) but
848 different d coordinates. The points with higher d must therefore be part of a surface protrusion and thus
849 $S(d, u, v)$ cannot be a $S_{\text{ref}}(d_{\text{ref}}, u, v)$. A suitable $S_{\text{ref}}(d_{\text{ref}}, u, v)$ can thus be found as the (u, v) parametrized
850 2D 'baseline' surface, $d_{\text{ref}} = f_{\text{smooth}}(u, v)$ to a 2D adaptation of the asymmetric least squares problem
851 (ALS)[109]; $d_{\text{ref}} = \arg\min_z\left\{\sum(w_{uv}(d_{uv}^{S'(d,u,v)} - d_{uv})^2 + \lambda\sum_{uv}(\Delta d_{uv})^2\right\}$ with asymmetric weights, $w_{uv}: w_{uv} = p$ if
852 $d_{uv}^{S'(d,u,v)} > d_{uv}$ and $w_{uv} = 1 - p$ otherwise. The regularization parameter, λ controls the contribution of the
853 Laplacian $\Delta d_{uv} = \nabla^2 d_{uv}$. The solution is a surface intermediate between a (u, v)-parameterization
854 approximation, $d_{uv}^{S'(d,u,v)} = f(u, v)$ of the topographic surface $S(d \approx f(u, v), u, v)$ and the flat 2D-plane $(d = $
855 $0)$ (Extended Fig. 3a). The input 1024x512 pixels approximation $(d_{uv}^{S'(d,u,v)})$ was computed as an image by
856 extending a vertical ray upwards at each (u, v) pixel and setting the image pixel value as the longest
857 contiguous stretch of the topographic binary. We downsample $d_{uv}^{S'(d,u,v)}$ 8x to 128x64 pixels for computational
858 efficiency and additional smoothness regularization and solve for d_{ref} by running 10 iterations of ALS[109] using
859 $p = 0.25, \lambda = 1$. The solution, d_{ref} is resized back to 1024x512 pixels. The height, h of $S(d, u, v)$ relative to
860 the inferred reference surface is the difference, $h = d - d_{ref}$ between a vertex's d coordinate and d_{ref} of the
861 matching point on $S_{\text{ref}}(d_{\text{ref}} = f_{\text{smooth}}(u, v), u, v)$ as found by interpolation.

863 Topography guided binary segmentation of protrusions
864

865 For cMCF binary segmentation of $S(d, u, v)$, the reference surface used is the 2d (u, v) plane,
866 $S_{ref}(d_{ref}, u, v) = S(d = 0, u, v)$ and the height is $h = d$. For more optimal segmentation, the reference surface,
867 $S_{ref}(d_{ref}, u, v)$ was inferred as above and the height is $h = d - d_{ref}$, relative to the matching point on
868 $S_{ref}(d_{ref}, u, v)$ with identical (u, v) coordinate. For both, the mean height, $\bar{h}$ is the threshold to give the initial
869 binary segmentation, $F_i(S(d, u, v)) = h \geq \bar{h}$. We postprocess by applying graph connected component
870 analysis to remove small segmented regions with surface area < 200 voxels^2; diffusing the result using two-
871 class labelspreading[110] with an affinity matrix, A, for 20 iterations, clamping ratio 0.99, and binarizing the label
872 probability with threshold of 0.25 at the start of each iteration. Lastly, any remaining small regions with surface
873 area < 500 voxels^2 was removed. The affinity matrix[69], A is a weighted sum ($\gamma = 0.9$) of an affinity matrix
874 based on geodesic distance, A_{dist} and one based on surface convexity, A_{convex}; $A = \gamma A_{dist} + (1 - \gamma)A_{convex}$
875 of $S(d, u, v)$. $A_{dist} = \begin{cases} e^{-D_{dist}^2/(2\mu(D_{dist})^2)} & i \neq j \\ 1 & i = j \end{cases}$ where D_{dist} is the pairwise Euclidean distance matrix between
876 two vertices i and j. $A_{convex} = \begin{cases} e^{-D_{convex}^2/(2\mu(D_{convex})^2)} & i \neq j \\ 1 & i = j \end{cases}$ where D_{convex} is the pairwise Cosine distance,
877 $\frac{(1-\cos(\theta_{ij}))}{2}$ matrix of the dihedral angle, θ_{ij} between the normal vectors at two vertices i and j. $\mu(D)$ denotes
878 the mean value of the entries of matrix D.
879

880 <u>Topography guided instance segmentation of protrusions</u>
881 Individual protrusions are segmented by identifying high curvature protrusive features and applying
882 connected components analysis. We compute the topographic mean curvature $H(S(d, u, v)) = -\frac{1}{2}\nabla \cdot \hat{n}$ with
883 the normal, $\hat{n}$ given by the unit gradient of the signed distance transform of the binary topographic volume of
884 the cell, $I_{binary}(V(d, u, v))$. We compute a binary subvolume restricted to the surface, $I_{surf}(V(d, u, v))$, the
885 intersection of the morphological dilation of $I_{binary}(V(d, u, v))$ with ball kernel size 2, and the morphological
886 erosion of $I_{binary}(V(d, u, v))$ with ball kernel size 2. To identify high curvature surface regions, $H_{high}(d, u, v)$
887 for lamellipodia, we concatenate $H(S(d, u, v))$ Gaussian smoothed with $\sigma = 1,3,5$ as a 3-dimensional feature
888 for all voxels in $I_{surf}(V(d, u, v))$ and apply Gaussian mixture model (GMM) clustering (# classes = 3), keeping
889 the class with the highest mean H. To identify $H_{high}(V(d, u, v))$ for blebs and filopodia which are circular and
890 smaller, we use $H(S(d, u, v))$ Gaussian smoothed with $\sigma = 1$ as a 1-dimensional feature for all voxels in
891 $I_{surf}(V(d, u, v))$ and apply kmeans clustering (# classes = 3), keeping the class with the highest mean H. For
892 efficiency, both GMM and kmeans clusterers are fitted on a random sampling of 10,000 surface voxels. Small
893 regions with < 500 connected voxels are removed. Connected component analysis labels each disconnected
894 region in $H_{high}(V(d, u, v))$ as individual protrusions, $I_{protrusions}(V(d, u, v))$. We expand labels by 3 voxels
895 and transfer the segmentation to the surface mesh, $S(d, u, v)$ by interpolation at the vertex coordinates,
896 $F_{protrusions}(S(d, u, v))$ for further surface-based processing. We first apply the binary protrusion
897 segmentation above, $B_{protrusion}(S(d, u, v))$ to $F_{protrusions}(S(d, u, v))$, taking the intersection and keeping
898 segmentations with size > 100 voxel^2 Cartesian 3D surface area. We diffuse segmentation labels with
899 labelspreading, clamping ratio 0.99 for 10 iterations, with affinity matrix A, $\gamma = 0.9$ as above. We do not
900 rebinarize the label probability at the start of each iteration. Finally, we apply $B_{protrusion}(S(d, u, v))$ to the
901 diffused segmentations to get the final instance segmentation labels, $F_{protrusions}(S(d, u, v))$.
902
903

904 <u>Direct 2D unwrapping of protrusion submeshes</u>
905
906 Segmented individual protrusions are open 3D surfaces with disk topology and can be directly unwrapped
907 into 2D if they possess no holes or handles and have one boundary. The genus, g of an open orientable
908 surface with b boundaries is computed from the Euler characteristic, $\chi = 2 - 2g - b = \#V - \#E + \#F$. Similar
909 to the spherical parameterization of closed 3D surfaces, the open 3D surface is first mapped conformally to
910 the unit disk then relaxed to get an equiareal disk parameterization.
911
912 *Quasi-conformal disk parametrization of genus-0 open surfaces.* We obtain a quasi-conformal map of an
913 open 3D surface to the unit disk by harmonic parametrization[111]. The boundary vertices are first mapped to
914 the boundary of the unit circle, whilst preserving edge length fractions. Interior vertices are then mapped to
915 the disk interior by solving Laplace's equation, $\nabla^2\phi = 0$.
916

917 *Equiareal disk parameterization by mesh relaxation.* We relax the conformal disk parametrization whilst
918 preserving the boundary topology using the area-preserving flow method[112]. We solve Poisson's equation to
919 compute the smooth vector field for diffusing the area distortion and explicit Euler integration to advect vertex
920 points iteratively with Delaunay triangle flips. The extent of area relaxation achieved is determined by the
921 mesh quality and number of vertices with respect to the extremity of local area distortion. In general, relaxation
922 was less stable compared to our relaxation for spherical surfaces above. For thin and long protrusions, prior
923 downsampling and uniform remeshing of the protrusion submesh was necessary to enable full area distortion
924 relaxation.

926 To convert a unit disk parameterization to an N x N pixel image, we 'square' the disk using the elliptical grid
927 mapping formula[113], multiply the resulting vertex coordinates by $N/2$ and interpolate the coordinates and
928 associated vertex quantities onto a N x N pixel integer grid. This gives similar results to but is significantly
929 faster than solving the Beltrami equation[94].

931 <u>Refinement of undersegmented blebs</u>

932 Given $S(x, y, z)$ and the vertex ids corresponding to protrusion i, (v_i) we first impute any small holes in the
933 segmentation; inner vertices not assigned to protrusion i but should be in order to ensure the protrusion
934 submesh, $S_{\text{protrusion}}(x, y, z)$ is a genus-0 open surface. We do this by applying graph connected component
935 analysis on the submesh formed by all vertex ids not part of protrusion i, $\{v\}\backslash\{v\}^i$. Any component with
936 number of vertices <10% the total surface area of $S(x, y, z)$ is assigned to protrusion i to form $\{v\}_{\text{impute}}^i$. The
937 submesh $S_{\text{protrusion}}(x, y, z)$ is formed from $\{v\}_{\text{impute}}^i$. We downsample $S_{\text{protrusion}}(x, y, z)$ by ¼ the number of
938 vertex points and remesh using ACVD as described above both for computational efficiency and to get a
939 higher quality mesh, $S_{\text{protrusion}}^{ds}(x, y, z)$ required for computing the intermediate equiareal disk
940 parameterization for a final square parameterization. $S_{\text{protrusion}}^{ds}(x, y, z)$ is directly unwrapped to a 2D 128 x
941 128 pixel square image as described above. Positive curvature 'seed' regions are identified by thresholding
942 the mean curvature mapped to 2D, $H\left(S_{\text{protrusion}}^{ds}(x, y, z)\right) > H_{thresh}$ with a global threshold and then applying
943 morphological closing, disk kernel radius 1 pixel. To classify regions as having negative, flat and positive
944 mean curvature, 3-class Otsu thresholding was applied to $H(S(x, y, z))$ to give two thresholds. All regions
945 with mean curvature greater than the higher threshold H_{thresh} were positive curvature. Undersegmented
946 blebs correspond to a binary composed of conjoined pseudo-circular regions. We use the gradient
947 watershed[114,115] on the Euclidean distance transform of the high curvature region binary to automatically
948 separate conjoined blebs without seed markers. Mesh matching and interpolation was used to map H and
949 segmentation labels between $S_{\text{protrusion}}(x, y, z)$ and $S_{\text{protrusion}}^{ds}(x, y, z)$. The refined segmentation were
950 mapped as seed labels from $S_{\text{protrusion}}(x, y, z)$ for every protrusion back to $S(x, y, z)$. The revised seed labels
951 were then diffused across $S(x, y, z)$ using the combined geometrical and convexity affinity matrix from above
952 for 10 iterations with $\alpha = 0.99$. The binary protrusion segmentation from above is applied, and any
953 segmentation with Cartesian 3D surface area < 10 voxels^2 removed to give the final refined protrusion
954 segmentation instances.

956 <u>Topography guided decomposition of cell surface</u>

958 The instance protrusion segmentation $F_{protrusions}(S(d, u, v))$ above assigns a unique protrusion label ID to
959 each vertex of the surface mesh, $S(d, u, v)$. We use this surface-based $F_{protrusions}(S(d, u, v))$ as seed labels
960 to partition the total internal cell volume into the volume space unique to each protrusion i, $V_{protrusion}^i(d, u, v)$
961 and the reference cortical cell volume, $V_{ref}(d, u, v)$. This is done in three parts; the construction of $V_{ref}(d, u, v)$,
962 volume propagation of $F_{protrusions}(S(d, u, v))$, and using the previous two parts to volumize individual
963 protrusions to obtain $V_{protrusion}^i(d, u, v)$ (Extended Fig. 4e-g).

965 *Construction of reference surface by imputation.* The reference surface with segmented protrusions removed,
966 $S_{\text{ref}}(d, u, v)$ is of the functional form $S_{\text{ref}}(d = f(u, v), u, v)$ with $f(\cdot)$ injective and thus can be described by
967 $d_{\text{ref}} = d = f(u, v)$ only. This is a 2D image with d_{ref} as the pixel value. We impute the subset of pixels with
968 missing values corresponding to the removed surface protrusions from pixels with known d_{ref} using the fast
969 marching image inpainting[116] implemented in the Python OpenCV library with an inpaint radius = 1 (Extended

Fig. 4e). The inpainted surface, $S_{ref}(d_{ref} = f_{inpaint}(u,v), u, v)$ is used to construct the binary reference cortical volume, $I_{binary}\left(V_{ref}(d, u, v)\right)$ which is 1 for all voxels whose $d < d_{ref} = f_{inpaint}(u,v)$.

Volume propagation of surface-based instance protrusion segmentation. The surface-based protrusion segmentation, $F_{protrusions}(S(d,u,v))$ is converted to voxel-based by setting the value of the voxels corresponding to the integer discretized $S(d,u,v)$ coordinates to the matching protrusion label ID. We expand the labels by 3 voxels using the Python Scikit-Image *skimage.segmentation.expand_labels* function and mask with the topographic binary cell volume $I_{binary}(V(d,u,v))$ to get the initial topographic volume protrusion segmentation, $I_{protrusions}(V(d,u,v))$ with only the surface of protrusions labelled. We apply marker watershed segmentation slice-by-slice to propagate labels laterally into the protrusion volume within a slice and labels from previous slices, from the top, $d = +D_{out}$ to the bottom, $d = -D_{in}$ of the topographic volume. At a slice $d = d_0$, we use the Euclidean distance transform of $I_{binary}(V(d = d_0, u, v))$ for watershed with the seed markers given by the labels of the previous slice, $d = d_0 + 1$ combined with the current labels of $I_{protrusions}(V(d = d_0, u, v))$ at slice $d = d_0$. In combining labels, the labels of the previous slice $d = d_0 + 1$ takes precedence and overwrites the label of $I_{protrusions}(V(d = d_0, u, v))$. The result, $I_{protrusions}(V(d,u,v))$ assigns a protrusion ID to all voxels in the entire cell volume $I_{binary}(V(d,u,v))$ (Extended Fig. 4f).

Volumization of individual protrusions. The binary reference cortical volume, $I_{binary}\left(V_{ref}(d,u,v)\right)$ is applied to exclude all cortical volume voxels in the watershed depth propagated $I_{protrusions}(V(d,u,v))$. We then apply connected component analysis to each unique protrusion label in $I_{protrusions}(V(d,u,v))$ and keep for each label, the largest contiguous volume region. The resulting $I_{protrusions}(V(d,u,v))$ is the final volume segmentation of all individual protrusions. For each unique protrusion, we generate a closed surface mesh by marching cubes. If the marching cubes mesh has > 1000 vertices, we downsample the mesh by a factor of 4 and remesh with ACVD. This last step is to keep the combined number of vertices across all protrusions and the reference surface reasonable for rendering and processing.

<u>Direct Cartesian 3D decomposition of cell surface</u>
For each segmented protrusion i, we construct the Cartesian 3D submesh, $S_{protrusion}^i(x,y,z)$. We find the set of vertices on the open boundary, $\{v\}_{boundary}$ using the Python libigl library function, *igl.boundary_loop*, compute the mean of these points, $\bar{v}_{boundary}$ and form a submesh, $S_{cap}(x,y,z)$ with $\{v\}_{boundary}$ and $\bar{v}_{boundary}$. We upsample S_{cap} by successive mesh subdivision 3 times, each time replacing a triangle face by the four new faces formed by adding vertices at the midpoint of every edge, giving a mesh with $\approx 4^3 = 64$ times more vertices. Finally we solve the Possion problem[72] to find the vertex coordinates of S_{cap} corresponding to the least bending energy. $S_{protrusion}^i(x,y,z)$ and $S_{cap}(x,y,z)$ are merged to form a closed surface mesh of protrusion i. Similarly $S_{cap}(x,y,z)$ is merged with the residual reference surface with segmented protrusions removed, $S_{ref}(x,y,z)$ to impute and close the hole left by protrusion i.

<u>Conformalized mean curvature flow (cMCF) for flattening topographic surfaces, $S(d,u,v)$</u>
$S(d,u,v)$ are open surfaces. Application of cMCF[49], which is designed for closed surfaces, maps $S(d,u,v)$ onto the 2D plane as an elliptical disk and in the limit to a point. We want the flow to converge to the planar (u,v) rectangle. To do so, we impose additional no-flux constraints in the u-, v- directions on the boundary, ∂S but allow flow in the depth, d direction by adding to the right hand side of the cMCF equation an external force term that applies only in the u-, v- directions. In interior vertices, the flow follows the standard cMCF.

$$cMCF_{topo} := \begin{cases} \left(\mathbf{M}_t^{boundary} - \delta\mathbf{L}_0^{boundary}\right)v(t+\delta t) = \mathbf{M}_t^{boundary}v(t) + \underbrace{\left[\mathbf{0}_d \Big| \left(-\delta\mathbf{L}_0^{boundary}v(t)\right)_{uv}\right]}_{to\ ensure\ no\ flux\ in\ u,v\ direction}, & \text{on } \partial S \\ \left(\mathbf{M}_t^{mesh} - \delta\mathbf{L}_0^{mesh}\right)v(t+\delta t) = \mathbf{M}_t^{mesh}v(t), & \text{on } S\backslash\partial S \end{cases}$$

where on the boundary, ∂S we use the mass, $\mathbf{M}_t^{boundary}$ and Laplacian, $\mathbf{L}_0^{boundary}$ matrix defined for a 2D line and $\mathbf{M}_t^{mesh}$, $\mathbf{L}_0^{mesh}$ is the mass and Laplacian matrices defined for a 3D triangle mesh. $[\mathbf{A}|\mathbf{B}]$ is used to denote the augmented matrix formed by appending the columns of matrix $\mathbf{A}$ and $\mathbf{B}$. We solve for the vertex position at the next timepoint $v(t+\delta t)$ as with cMCF by direct matrix inversion.

Surface curvature measurement

The mean curvature, H of a 3D surface was measured as the divergence of $\hat{n}$, the unit surface normal[103], $H = -\frac{1}{2}\nabla \cdot \hat{n}$. The surface mesh is voxelized to a binary volume, B and $\hat{n}$ is computed as the gradient of the signed distance transform of B with the Euclidean distance metric. H computed in this manner as opposed to from the mesh directly using discrete differential geometry[103] or quadric plane fitting[117] which is less affected by the number of mesh vertices or the mesh quality.

Mesh quality measurement

The radius ratio $= 2\frac{r_{in}}{r_{circ}}$, defined as twice the ratio between inradius and circumradius was used to measure the face quality for a triangle mesh in Extended. Fig. 2,3. It is a mesh quality measure in the sense that the radius ratio obtains its maximum value of 1 for an equilateral triangle; the shape which jointly maximizes all internal angles and gives the best conditioning number for the mesh Laplacian matrix[118].

Surface rendering

Triangle meshes were exported from Python using the Python Trimesh library into .obj mesh files and visualized in MeshLab[119]. Volumetric images were rendered in Fiji ImageJ through the volume viewer plugin, and intensities were contrast enhanced for inclusion in the figures using Microsoft PowerPoint. The local surface maximum intensity projection image of Fig. 4c was produced by extending z-axis (depth) rays at every xy pixel, and taking the maximum intensity of voxels within ± 9 voxels ($\pm 1\mu$m) of the cell surface.

Datasets

Cell morphology validation dataset

To validate u-Unwrap3D (Fig. 2,3 and Extended Fig. 2-4), we used 66 cell surfaces segmented and surface protrusions classified using u-Shape3D and acquired from high resolution light sheet microscopy[4,120] as previously described[45]. The surfaces include 19 MV3 melanoma cells expressing Lifeact-GFP showing blebs, 38 dendritic cells expressing Lifeact-GFP showing lamellipodia, and 9 human bronchial epithelial (HBEC) cells expressing Tractin-GFP. We applied u-Unwrap 3D to these datasets with the following parameters for each step: for Step 1, cMCF with maximum iterations = 50, $\delta_t = 5 \times 10^{-4}$, stopping threshold, $\Delta_{thresh} = 1 \times 10^{-5}$ for blebs, $= 1 \times 10^{-5}$ for lamellipodia, $= 1 \times 10^{-4}$ for filopodia, $S_{\text{ref}}(x,y,z)$ mesh voxelization with morphological dilation and erosion with ball kernel radius 5 voxels, Gaussian smoothing $\sigma = 1$ of binary volume and initial $S_{\text{ref}}(x,y,z)$ meshing with marching cubes at isovalue 0.5, ACVD remeshing with number of clusters = 90% the number of vertices in the marching cubes mesh; for Step 3, area distortion relaxation with maximum iterations = 100, $\delta_t = 0.1$, stepsize $\varepsilon = 1$ and if equiareal was not achieved, repeat relaxation with a slower $\delta = 5 \times 10^{-3}$; for Step 4, the mean curvature of $S_{\text{ref}}(x,y,z)$ was used as the weight for determining the unwrapping axis, and a 1024 x 512 pixel (u,v) grid; for Step 5, an upsampling factor of 3 for binary voxelization, $\alpha = 0.5$ voxel steps, a specified $D_{in} = 40$ steps and 2D robust smoothing[121] with smoothing factor = 50 for each iteration; for Step 6, binarization of the topographic 3D mapped binary cell segmentation with a threshold of 0.5, then Gaussian smoothing $\sigma = 1$ and initial marching cubes meshing at isovalue 0.5, ACVD remeshing with number of clusters = 50% the number of vertices in the marching cubes mesh.

3D timelapse lightsheet imaging and analysis of blebs

Cell culture and timelapse imaging. All details of the cell line creation, culture and imaging of the MV3 GFP-expressing melanoma cell movie in Fig. 4 were previously published[38]. The movie is a total of 200 frames acquired at a frequency of 1.21 s per frame. Each frame is a 104 x 512 x 512 size 3D volume with a voxel resolution of 0.300 x 0.104 x 0.104 μm.

067

068 *Cell segmentation and surface meshing.* The 200 timepoints were spatiotemporally registered volumetrically
069 to the first timepoint, $t = 0$ as previously described[38]. The cell surface at $t = 0$ was segmented using a multi-
070 level method[122] involving local contrast enhancement, deconvolution and edge enhancement and surface
071 meshed as described above to obtain $S^{t=0}(x, y, z)$. Images were deconvolved using the Wiener-Hunt
072 deconvolution approach[123] with our previously published point-spread function[45].The surface mesh at all
073 subsequent timepoints, $S^t(x, y, z)$ were reconstructed using the non-rigid registration deformation field from
074 volumetric registration[38]. The vertex Septin intensity was calculated by extending from the surface a trajectory
075 to an absolute depth of 1 μm along the steepest gradient of the distance transform to the mesh surface, and
076 assigning the 95th percentile of intensity sampled along that trajectory to the originating vertex to capture the
077 systematically brightest accumulation of Septin signal in the cortical shell. The raw Septin intensity suffers
078 decay from bleaching. We simultaneously normalized and corrected the vertex Septin intensity by computing
079 a normalized Septin intensity as the raw intensity divided by the mean Septin intensity in the whole cell
080 volume at each timepoint.

081

082 *u-Unwrap3D analysis.* We computed a mean surface mesh, $\bar{S}(x, y, z)$ from all $S^t(x, y, z)$ as the input surface
083 to u-Unwrap3D. This was done by surface meshing the mean binary volume over all binary voxelizations of
084 individual $S^t(x, y, z)$ at an isovalue of 0.5. u-Unwrap3D was applied to $\bar{S}\{(x, y, z)$ to create a common static
085 (d, u, v) coordinate space that all $S^t(x, y, z)$ is mapped to in Step 5 of u-Unwrap3D to generate $S^t(d, u, v)$. u-
086 Unwrap3D was run with the same parameters for all steps as for blebs in the validation dataset, except for
087 the following modifications: step 1, the same automatic stopping iteration number but +5 steps, and ACVD
088 with 10% of the marching cubes mesh to get a smoother $S_{ref}(x, y, z)$; step 4, a smaller 512 x 256 pixel size
089 (u, v) grid and not using the unwrapping axis inferred by curvature-weighted PCA - this axis passed through
090 a large bleb and affected tracking; step 5, $D_{in} = 96$ steps - a total of 5μm. Topographic cMCF with the robust
091 mesh Laplacian[102], mollify factor $= 1 \times 10^{-5}$, $\delta_t = 5 \times 10^4$ was applied to each $S^t(d, u, v)$ for 10 iterations to
092 compute the corresponding $S^t(u, v)$.

093

094 *Bleb segmentation and tracking.* Blebs were segmented from $S(d, u, v)$ at every timepoint using the instance
095 segmentation algorithm with refinement for undersegmented blebs as described above. In computing the
096 binary protrusion segmentation we use a downsampling factor of 4 due to the smaller 512 x 256 pixel (u, v)
097 grid and diffuse the segmentation for 5 iterations as the blebs were smaller than the validation dataset. The
098 segmented 512 x 256 (u, v) bleb images, $F_{bleb}(S(u, v))$ were padded 50 pixels on all four sides respecting
099 spherical topology. This is done by periodic padding along the u- axis. For the v- axis, we pad the top of the
100 image by reflecting the pixels with respect to the first image row (i.e. all pixels in row 2 to row 51) and then
101 flipping in the u- axis. Similarly, the bottom is padded by refecting the pixels with respect to the last image
102 row (i.e. all pixels in row 2 to row 511) and then flipping in the u- axis. For each unique bleb in every timepoint,
103 we computed the bounding box of the bleb given by top left, (u_{min}, v_{min}) and bottom right (u_{max}, v_{max})
104 coordinates. The bleb bounding boxes were tracked using an optical flow assisted bounding box tracker[77].
105 Boxes were linked over time into tracks using bipartite matching and the intersection over union (IoU>0.25
106 for valid match) of bounding boxes as the distance function between pairs. To handle large changes in box
107 size, the matching between the current and next frame was carried out on the predicted bounding box
108 coordinates by local optical flow[124]. Optical flow was computed using the mean curvature, $H(S(u, v))$ after
109 rescaling $H(S(u, v))$ to be an 8-bit grayscale image using the global minimum and maximum curvature values
110 over time. In case of temporary occlusion or missed segmentation, any non-matched blebs were propagated
111 for up to 5 frames (6s) using the estimated optical flow before track termination. Tracks with > 5 frames (6s)
112 and a mean positive curvature, $H > 0.1$ μm^{-1} were retained as bleb tracks. The coordinates of retained tracks
113 was corrected to account for the initial padding of 50 pixels. To remove erroneous and duplicated tracks, we
114 uniquely match every segmented bleb in each timepoint to a track by IoU. For each track, we then computed
115 the fraction of its lifetime that could be matched to a bleb and removed all tracks for which this proportion
116 was < 50%. Lastly for each track we checked for sudden changes in the bounding box area, which was
117 indicative of an erroneous bounding box in need of substitution by an inferred corrected bounding box. We
118 applied this procedure to each track in order to construct the timeseries of the bounding box area over the
119 track lifetime and compute a smooth reference timeseries using the central moving average with a window
120 of 3 frames. The bounding box at a timepoint is erroneous if the instantaneous difference between the raw
121 and smooth bounding box area > 500 pixel^2 (the mean (u, v) bleb box area is 361 pixel^2). The coordinates of
122 a corrected bounding box is inferred from non-erroneous bounding boxes by interpolation using a linear spline.
123 The tracks that remained fully in-focus over its lifetime were retained for analysis.

124

125 *Bleb timeseries extraction.*

126 We detected (u, v) pixels on blebs by labelling spatially contiguous areas of positive mean curvature based
127 on 3-class Otsu thresholding defining positive, flat or negative curvature. The largest connected component
128 within a bleb bounding box was defined as on-bleb and the remainder area within the bounding box as off-
129 bleb. We extracted distortion-corrected average timeseries of bleb area, mean curvature and septin intensity,
130 that is of a scalar quantity, F by observing that the mean of F over a Cartesian 3D surface area is equivalent
131 to computing a weighted mean over the equivalent (u, v) area, $\frac{\iint_S F(S(x,y,z))\, dS}{\iint_S dS} = \frac{\iint_S F(S(u,v))\, dA\, dudv}{\iint_S dA\, dudv}$. The weight,
132 dA is the magnitude of the differential area element, $dA = \left| \frac{\partial S}{\partial u} \times \frac{\partial S}{\partial v} \right|$ described above.

133
134 *Bleb event alignment.*
135 Individual blebbing events were detected within a track by applying peak finding after central moving
136 averaging of bleb area timeseries with a window of 3 timepoints. A peak was defined as having a prominence >
137 0.5 and separated from a neighboring peak by at least 3 timepoints. Individual bleb event timeseries were
138 constructed and temporally aligned using the detected timepoint of maximal bleb area as timepoint 0 and
139 taking a window of 14 timepoints on either side (a total 29 timepoints, 35 s).

140
141 3D timelapse lightsheet imaging and analysis of ruffles

142 *Cell culture and timelapse lightsheet imaging.* SU.86.86 cells were purchased from American Type Culture
143 Collection (CRL-1837). The cells were transfected with integrating lentiviral plasmids carrying genes for
144 myristoylated CyOFP1 and Tractin-mEmerald. The cells were cultured in RPMI medium supplemented with
145 10% fetal bovine serum and 1% anti-anti (Gibco 15240062), at 37°C in a humidified incubator and 5%
146 CO_2. SU.86.86 cells were imaged on fibronectin-coated coverslips on a custom axially swept light sheet
147 microscope[125]. The microscope detection system comprises a 25X NA1.1 water immersion objective (Nikon,
148 CFI75 Apo, MRD77220) and a 500mm tube lens. The illumination was done through a 28.6X NA0.66 water
149 immersion objective (Special Optics, 54-10-7). The movie analysed in Fig.5 is a total of 30 frames acquired
150 at a frequency of 2.27 s per frame. Each frame is a two-channel 151 x 1024 x 1024 size 3D volume with a
151 voxel resolution of 0.300 x 0.104 x 0.104 μm.

152

153

154 *Cell segmentation and surface meshing.* All timepoints were rigid registered volumetrically to the first
155 timepoint, $t = 0$ to compensate for drift. The CyOFP1 image was also rigid registered to the Tractin-
156 mEmerald in each timepoint. For every timepoint, the volumetric image was segmented using a multi-level
157 method[122] involving local contrast enhancement, deconvolution and edge enhancement and surface meshed
158 as described above to obtain the surface mesh, $S(x, y, z)$. The vertex Tractin-mEmerald and CyOFP1
159 intensities were calculated by extending a trajectory to an absolute depth of 1 μm along the steepest gradient
160 of the distance transform to the mesh surface, and taking the mean intensity along the trajectory.

161
162 *u-Unwrap3D analysis.* The first timepoint surface mesh was used as the input $S(x, y, z)$ to u-Unwrap3D to
163 create a common static (d, u, v) coordinate space that the surface meshes from all timepoints is mapped to
164 in Step 5 of u-Unwrap3D to generate $S^t(d, u, v)$. We use Unwrap-3D with the following parameters for each
165 step: Step 1, cMCF with maximum iterations = 50, $\delta_t = 1 \times 10^{-5}$, automatic stopping threshold, $\Delta_{thresh} = 5 \times$
166 10^{-5}, $S_{ref}(x, y, z)$ mesh voxelization with morphological dilation and erosion with ball kernel radius 5 voxels,
167 Gaussian smoothing $\sigma = 1$ of the binary volume and initial marching cubes meshing at isovalue 0.5, ACVD
168 remeshing with number of clusters = 10% the number of vertices in the marchin cubes mesh, and further
169 volume constrained Laplacian mesh smoothing[126] with implicit time integration, time step 0.5 for 15 iterations;
170 for Step 3 area distortion relaxation with maximum iterations = 100, $\delta_t = 0.1$, stepsize $\varepsilon = 1$; for Step 4 we
171 use the binary positive curvature region of $S_{ref}(x, y, z)$ given by 3-class Otsu thresholding as the weight for
172 determining the unwrapping axis, and use a 1025 x 512 pixel (u, v) grid; for Step 5, for outwards propagation,
173 an upsampling factor of 3 for binary voxelization, $\alpha = $ minimum of 0.5 and $\frac{1}{2}(\Delta u + \Delta v)$ voxel -steps where
174 Δu, Δv is the mean Cartesian 3D distance of traversing one pixel in u, v directions and a separable 1D
175 uniform box filter smoother with a window 5 pixels; for inwards propagation, we use active contour cMCF with
176 $\delta_t = 5 \times 10^{-4}$, and robust mesh Laplacian[102], mollify factor 1×10^{-5} for better numerical stability with the
177 (u, v) parameterized $S_{ref}(x, y, z)$ converted into a triangle mesh by triangulating the quadrilateral pixel
178 connectivity and inserting additional triangles to 'stitch' the image boundaries into a spherical topology, (note
179 the latter stitching requires an even number of columns after discounting that the last column is the same as

180   the first column, hence a 1025 x 512 grid) and D_{in} = (maximum internal distance transform value) / α steps;
181   for Step 6, marching cubes meshing of the topographic 3D mapped binary cell segmentation at isovalue 0.5
182   following Gaussian smoothing $\sigma = 1$, ACVD remesh with number of clusters = 50% the number of vertices in
183   the marching cubes mesh and retaining the largest connected component mesh. Topographic cMCF with
184   robust mesh Laplacian[102], mollify factor $= 1 \times 10^{-5}$, $\delta = 5 \times 10^4$ was applied to each $S(d, u, v)$ for 10
185   iterations to compute the corresponding $S(u, v)$.

187   *Optical flow ROI tracking.* We used motion sensing superpixels (MOSES)[83,84] in dense tracking mode which
188   automatically monitors the spatial coverage of ROIs and introduces new ROIs dynamically to ensure uniform
189   spatial tracking at every timepoint. We partitioned the image with an initial user-specified 1000 non-
190   overlapping rectangular regions-of-interest (ROI). Each ROI was tracked over time by subsequently updating
191   its centroid by the median optical flow[124] within the ROI. Optical flow was computed from the Traction/CyOFP1
192   (TC) signal after rescaling $TC(S(u, v))$ to be an 8-bit grayscale image using the video minimum and maximum
193   TC values.

195   *ROI timeseries extraction and cross-correlation.* Distortion-corrected average timeseries of mean
196   Tractin/CyOFP1 (TC) and H were computed for a track using the same weighted mean as for blebs. A square
197   bounding box of the mean MOSES ROI width centered at the track (u, v) coordinate was used to sample the
198   scalar values at each timepoint. The distortion-corrected timeseries can be treated as standard 1D timeseries.
199   The 1D normalized cross-correlation was thus computed between the distortion-corrected TC and H
200   timeseries for individual tracks without modification. ROI cross-correlation curves were averaged at all time-
201   lags to derive the mean and 95% confidence interval ROI cross-correlation curve. A deviation of the curve
202   greater than the 95% confidence interval at a time lag of 0 indicated significant instantaneous correlation.

204   *Retrograde actin flow and mean ruffle travel speed.* Computing the speed histogram with 25 bins and speed
205   range 0-10 μm/min showed a slow and fast population (Fig. 5d). We inferred the mean speed of the two
206   populations as the two thresholds generated by 3-class Otsu thresholding. The lower and faster of the
207   thresholds are the mean speed of retrograde actin flow and ruffles respectively.

209   *Cross-correlation and curvature relationship.* We computed the continuous relationship of mean curvature,
210   H and the lag 0 cross-correlation of TC and H (Fig. 5e) over ROI tracks using kernel density. Gaussian kernel
211   density with a bandwidth set by Scott's rule was used to derive the joint density distribution of H and cross-
212   correlation i.e. $p(X, Y)$, with X: H, Y: cross-correlation over the closed intervals $X \in [-0.2, 0.6]$ and $Y \in$
213    $[-1, 1]$. The continuous relationship is then given by the marginal expectation with capital denoting the
214   random variable and $\mathbb{E}[\cdot]$ the expectation operator, $\mathbb{E}[Y|X = x] = \int Y \, p(Y|X = x)dY = \int Y \frac{p(X,Y)}{p(X)} dY =$
215    $\frac{\int Y \, p(X,Y) \, dY}{\int p(X,Y)dY}$ with standard deviation equivalently defined as the square root of the variance,
216    $\mathbb{E}[(Y - \bar{Y})^2|X = x] = \mathbb{E}[Y^2|X = x] - \mathbb{E}[Y|X = x]^2$. The evaluation of the integrals uses 100 bins for both H and
217   cross-correlation.

a

Overview of u-Unwrap3D

u-Unwrap3D operates on surface meshes

Volume intensities := $I_i(V(x,y,z))$ *Binary volumes* := $I_i(V(x,y,z))$ *Surface signals* := $F_i(S(x,y,z))$

Key algorithmic steps

$F_i(S(x,y,z))$

(genus-X)

Input surface
$S(x,y,z)$

$\mathbf{v}_{t+1} = (\mathbf{M}_t - \delta\mathbf{L}_0)^{-1}\mathbf{M}_t\mathbf{v}_t$

Step 1. Iterative conformalized curvature smoothing

$F_i(S_{\mathrm{ref}}(x,y,z))$

(genus-0)

Reference surface
$S_{\mathrm{ref}}(x,y,z)$

Step 2.
Quasi-conformal spherical mapping

$\lambda_t = \mathbf{Area}_{\mathrm{ref}}/\mathbf{Area}_t$
$\nabla^{\mathrm{area}}\mathbf{v}_t = -\nabla\log\lambda_t$
$\mathbf{v}_{t+1} = (\mathbf{M}_t-\delta\mathbf{L}_t)^{-1}\mathbf{M}_t(\mathbf{v}_t+\epsilon\nabla^{\mathrm{area}}\mathbf{v}_t)$
$\mathbf{v}_{t+1} \leftarrow \mathbf{v}_{t+1}/\|\mathbf{v}_{t+1}\|$

Step 3. Iterative area distortion relaxation

$F_i(S^2_\Omega(x,y,z))$

Quasi-equiareal spherical reference surface
$S^2_\Omega(x,y,z)$

$F_i(S^2_Q(x,y,z))$

Quasi-conformal spherical reference surface
$S^2_Q(x,y,z)$

Step 4.
UV mapping

$F_i(S(u,v))$

$\Phi(x,y,z)$ = signed distance function
$\mathbf{v}_{d+1}(x,y,z) = \mathbf{v}_d(x,y,z)+\alpha\nabla\Phi_d(x,y,z)$

Step 5. Scan $S(u,v)$ surface to construct $D \times U \times V \times 3$ (x,y,z) coordinate lookup table and remap $I_i(V(x,y,z))$ intensities into topography space

uv-unwrapped reference surface
$S(u,v)$

$I_i(V(d,u,v))$
remapped raw intensity

remapped binary segmentation

remapped topographic volumes
$V(d,u,v)$

$F_i(S(d,u,v))$

Step 6.
remesh/resegment and map

topographic surfaces
$S(d,u,v)$

b

Special case: Direct unwrapping 3D surfaces (genus-0 mesh)

$F_i(S(x,y,z))$

(genus-0)

H, [1/um]

Input surface
$S(x,y,z)$

Step 1.
Quasi-conformal spherical mapping

$F_i(S^2_Q(x,y,z))$

Quasi-conformal sphere
$S^2_Q(x,y,z)$

Step 2.
Iterative area distortion relaxation

$F_i(S^2_\Omega(x,y,z))$

Quasi-equiareal sphere
$S^2_\Omega(x,y,z)$

Step 3.
UV mapping

$F_i(S(u,v))$

uv-unwrapped surface
$S(u,v)$

219
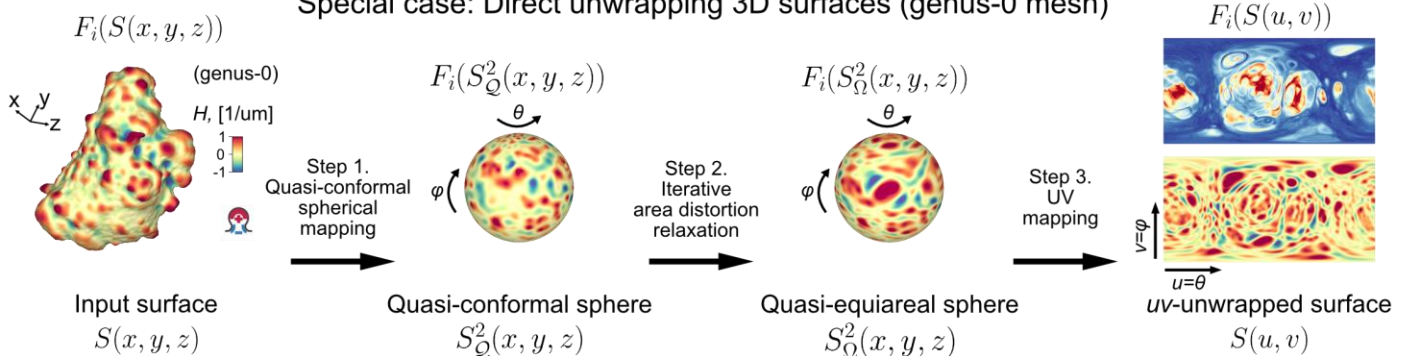
Figure 1. Overview of the surface-guided computing framework u-Unwrap3D. a) Overview of the 6 key steps to map an input genus-X Cartesian 3D surface, $S(x, y, z)$ such as that obtained from surface meshing the binary segmentation of an input 3D volume image, I_i, and associated scalar measurements, $F_i(S(x, y, z))$, via a smooth genus-0 reference surface, $S_{\text{ref}}(x, y, z)$, into any of three additional representations; topographic 3D surface, $S(d, u, v)$, 3D sphere, $S^2(x, y, z)$ and 2D plane, $S(u, v)$. $\mathbf{v}$ denotes mesh vertex coordinates, $\mathbf{M}$ is the mesh mass matrix, $\mathbf{L}$ is the mesh Laplacian matrix, ϵ the step size of area-distortion relaxation, α the step size (in pixels) of the propagation distance, d the topographic depth (in pixels) and t the iteration number. c) Steps to directly remap input genus-0 surfaces without need for a reference surface. In the figure, $S(\cdot), V(\cdot)$ denote surface and volume geometries, respectively, in either Cartesian 3D, topographic 3D, or radius-standardized 3D spherical coordinates; $F_i(S(\cdot))$ and $I_i(V(\cdot))$ denote surface or volumetric signals as a function of a particular surface or volume geometry. H and K denote mean and Gaussian curvatures respectively.

a

$F_i(S(x,y,z))$ (genus-X) H, [1/um]

3D Input surface, $S(x,y,z)$

Conformalized mean curvature flow

$F_i(S_{\text{ref}}(x,y,z))$ (genus-0)

3D reference surface, $S_{\text{ref}}(x,y,z)$

Direct spherical map (genus-0)

Coordinate lookup & interpolation

Spherical map

$F_i(S(d,u,v))$

3D Topography surface, $S(d,u,v)$

Depth propagation

$F_i(S(u,v))$

$v=\varphi$

$u=\theta$

2D UV image, $S(u,v)$

UV mapping

$F_i(S_\Omega^2(x,y,z))$

θ

φ

3D sphere, $S_\Omega^2(x,y,z)$

b

3D volume image $I_i(V(x,y,z))$

d v u

3D topography image $I_i(V(d,u,v))$
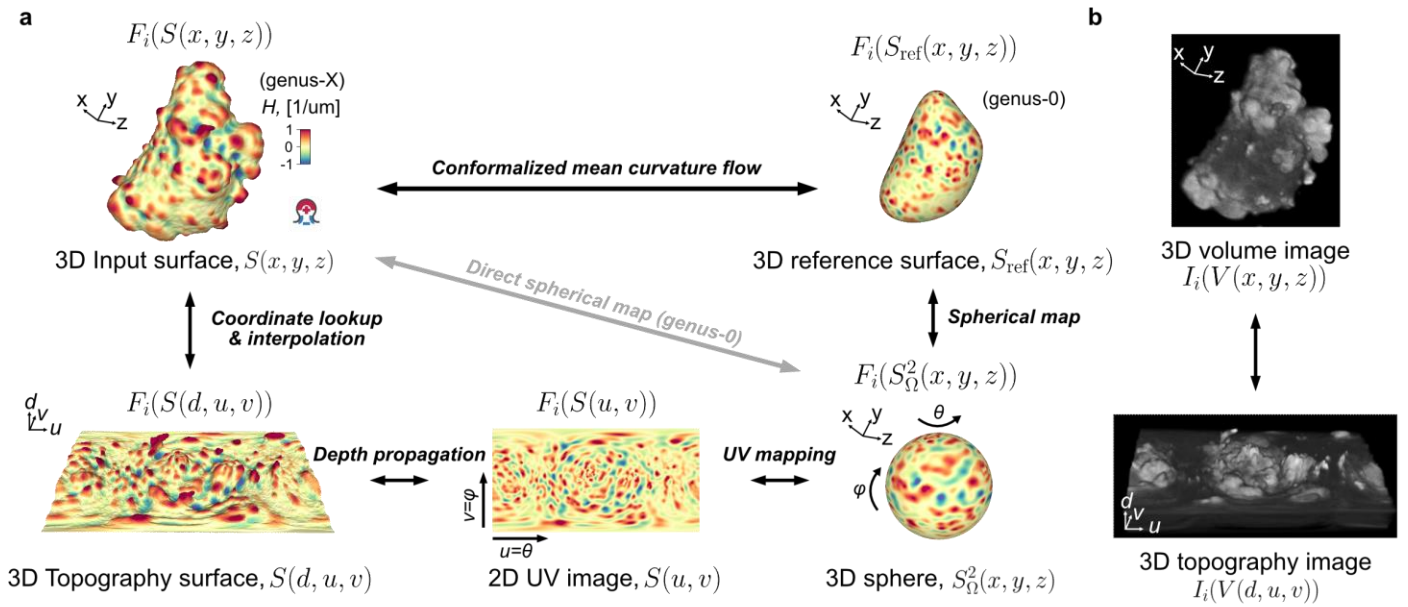
c

$F_i(S(x,y,z))$	$F_i(S_{\text{ref}}(x,y,z))$	$F_i(S_\Omega^2(x,y,z))$	$F_i(S(u,v))$	$F_i(S(d,u,v))$
Bleb (genus-577) #V: 324,130 #F: 650,588	(genus-0)			
Lamellipodia (genus-7) #V: 163,646 #F: 327,316	(genus-0)			
Filopodia (genus-2694) #V: 380,922 #F: 772,900	(genus-0)			

d

	Bleb	Lamellipodia	Filopodia
$I_i(V(x,y,z))$			
$I_i(V(d,u,v))$			

234
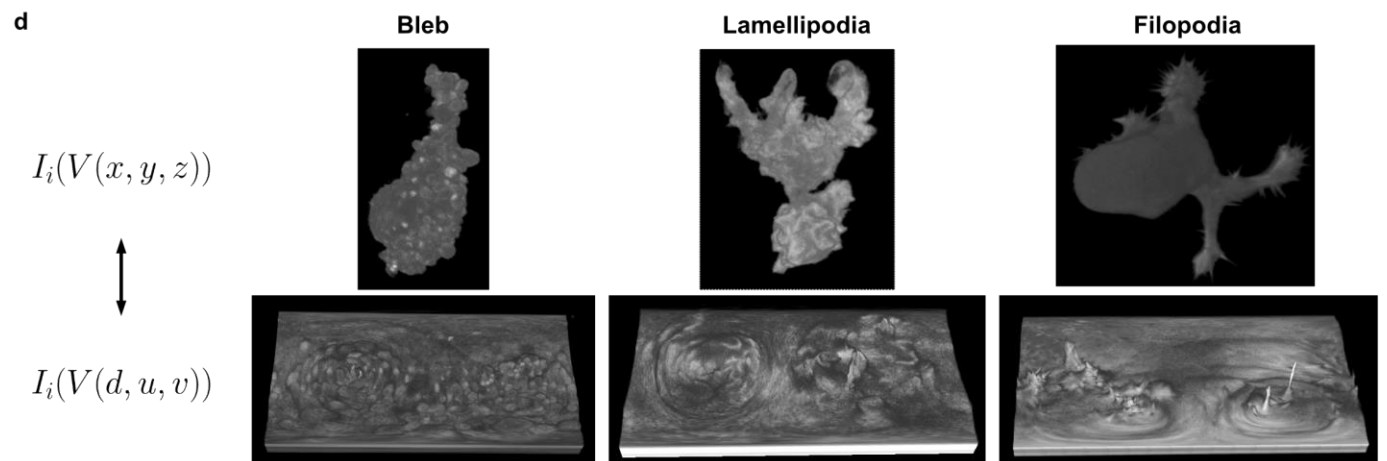
Figure 2. u-Unwrap3D generates a spectrum of equivalent data representations for surface-guided computing. a) Summary of the bijective mappings between the 5 equivalent surface mesh representations generated by u-Unwrap3D. Black bidirectional arrows indicate the mapping algorithms between representations discussed in the text. Grey arrow indicates the direct spherical mapping applicable when the input mesh is genus-0. **b)** u-Unwrap3D also enables bidirectional mapping of volumetric information between a Cartesian and topographic space relative to a genus-0 reference surface. **c)** Gallery of equivalent surface representations generated on examples of cell surfaces with blebs, lamellipodia and filopodia. For visualization of the mappings, individual instances of morphological motifs detected by the software uShape3D are color-coded on surface representations. **d)** Gallery of equivalent volume representations for the same cells shown in c). Volume image intensities were visualized using ImageJ volume viewer and contrast-enhanced to better visualize fine protrusions (see Methods).

a Measuring relative height of protrusions

b Binary segmentation of protrusions

c Instance segmentation of protrusions

d Adjusted NMI : 0.65
Rand Index : 0.80

e

f Mapping of individual protrusions to 2D for targeted analysis

g Refine segmentations with 2D gradient watershed

Before Refine
Adjusted NMI : 0.57
Rand Index : 0.76
Total # (uShape3D) : 742
Total # (topography) : 124

After Refine
Adjusted NMI : 0.54
Rand Index : 0.67
Total # (uShape3D) : 742
Total # (topography) : 150

h Reference surface + protrusions decomposition

i topography guided vs 3D mesh processing volumizations

j Mapping topographic 3D signals to 2D

247

Figure 3. u-Unwrap3D enables segmentation and characterization of complex 3D surface morphologies. a) The d-coordinate of the topographic 3D surface directly measures the protrusion height $S(x, y, z)$ of the input surface relative to the reference surface $S_{\mathrm{ref}}(x, y, z)$. Here, $S_{\mathrm{ref}}(x, y, z)$ was optimized for delineating surface protrusions (see Extended Fig. 4a,b). **b)** Surface segmentation obtained by binary thresholding of the height measured relative to the optimized (blue) reference surface (see Extended Fig. 4c). Surface protrusions above the threshold are in red. **c)** Overview of an unsupervised pipeline to detect and segment protrusion instances by thresholding the topographic curvature and connected component labeling (see Extended Fig. 4d). Individual protrusions are uniquely colored. **d)** Comparison of the topography-guided protrusion segmentation with supervised uShape3D morphological motif detection. Individual protrusions are uniquely colored. Quantitative concordance was measured by adjusted normalized mutual information (NMI, 0-1) and Rand index (0-1). **e)** Comparison of the surface area of topography-guided (red bars) and uShape3D-based (grey bars) segmented protrusions plotted relative to the full reference surface (top) including the grey colored cortical surface and zoomed-in (indicated by the polygon) comparing only the surface area of segmented protrusions (bottom). **f)** Selective 2D unwrapping of 3 individual segmented protrusions labelled i-iii) into corresponding 2D disk and square representations for fine-grained segmentation of under-segmented protrusions **g)** Application of a watershed algorithm to the 2D representations refines under-segmented protrusions. The bijectivity of all intermediary mappings permits the representation of coarse- and fine-grained segmentations back on the 3D surface. Comparison of the final protrusion segmentation to the segmentation before refinement (see f)) and to the motifs detected by uShape3D. Quantitative concordance was measured by adjusted normalized mutual information (NMI, 0-1) and Rand index (0-1). **h)** Decomposition of an input Cartesian 3D surface (left) into reference cortical surface (grey colored) (middle) and individual meshes (uniquely colored) per segmented protrusion (right). The decompositions were guided by the topographic representations (bottom) (see Extended Fig. 4e-g). The decomposed surface meshes are closed and define individual volumes (i.e. volumized). **i)** Comparison of the surface area (left, labelled i) and volumes (middle, labelled ii) of individual protrusions computed from u-Unwrap3D topography guided from h) (x-axis) or standard 3D mesh processing (see Methods) volumized surface meshes (y-axis). Reconstructed reference cortical surface meshes without protrusions and colored by mean curvature using from u-Unwrap3D topography guidance (bottom) or standard 3D mesh processing (top) (right, labelled iii). Black triangles highlight mechanically implausible surface features left by standard 3D mesh processing. **j)** Illustration of the modified conformalized mean curvature flow (cMCF) to directly map topographic 3D surfaces and associated signals (here, segmented protrusions marked by unique colors) to the 2D plane, an optimal representation for tracking individual protrusions.

a

$S(x, y, z)$ $S(d, u, v)$ $S(u, v)$

Instance segmentation of blebs frame by frame from topography and map to plane

$F_i(S(d, u, v))$ $F_i(S(u, v))$

Mapping of (x,y,z) surface mean curvature to plane

Mapping of (x,y,z) septin intensity to plane

b

Bounding box tracking in 2D to track 3D blebs

$F_i(S(u, v))$ $F_i(S(x, y, z))$

Extended segmentation and bounding box overlay

2D Bleb bounding box tracks

remapped 3D Bleb bounding box tracks

c Annotation and removal of tracks in out-of-focus regions

$F_i(S(x, y, z))$

annotate region on mesh

out-of-focus

map annotation to (u,v)

$F_i(S(u, v))$

out-of-focus

$F_i(S(u, v))$

out-of-focus

Filtered 2D Bleb bounding box tracks

d

Mean Length: 27s

e

H autocorrelation peak: 30s

f Reconstructed average bleb and molecular signal timeseries

280

281

Figure 4. u-Unwrap3D enables tracking and characterization of blebs and associated signals. a) Individual blebs segmented in topographic 3D representation are mapped to Cartesian 3D for visualization and to the 2D plane for tracking (top). Individual blebs are uniquely colored. Mean curvature, H (middle) and normalized Septin intensities (bottom) are jointly mapped from Cartesian 3D to topographic 3D to the 2D plane. The Septin intensity is normalized at each time to the mean Septin intensity in the whole cell volume to correct for expression variation and photobleaching. **b)** Tracking of individual blebs using an optical flow-guided 2D bounding box tracker. The unwrapped (u, v) -map is padded on all four sides to capture the continuation of the spherical surface (dashed black lines). Because of the bijectivity between representations individual bleb bounding box tracks in 2D (middle) can be mapped to 3D (right). **c)** Bijective mappings enable the transfer of manually annotated out-of-focus in Cartesian 3D to the unwrapped (u, v) 2D plane to restrict intensity timeseries analyses to only the bleb tracks within the in-focus surface regions. The decay in image contrast with sample depth is shown in a maximum projection image of the first timepoint restricted to the segmented surface ± 1 µm (Methods). Scalebar: 10 µm. **d)** Histogram of the in-focus bleb track lengths (dashed line, mean length). **e)** Autocorrelation curves (mean ± standard deviation) of mean curvature, H and Septin computed from Cartesian 3D meshes. Dashed black line depicts the lag time of the first autocorrelation side lobe of mean curvature, H. **f)**. Average (mean ± s.e.m) time course of bleb surface area (top), mean curvature (on bleb, black; off bleb, orange, and Septin intensity (bottom) over a window 17.5s before to 17.5s after the timepoint of maximum bleb size used for alignment (n=545 bleb events from m=1 cell). A-D labels distinct phases of bleb-mediated curvature Septin recruitment.
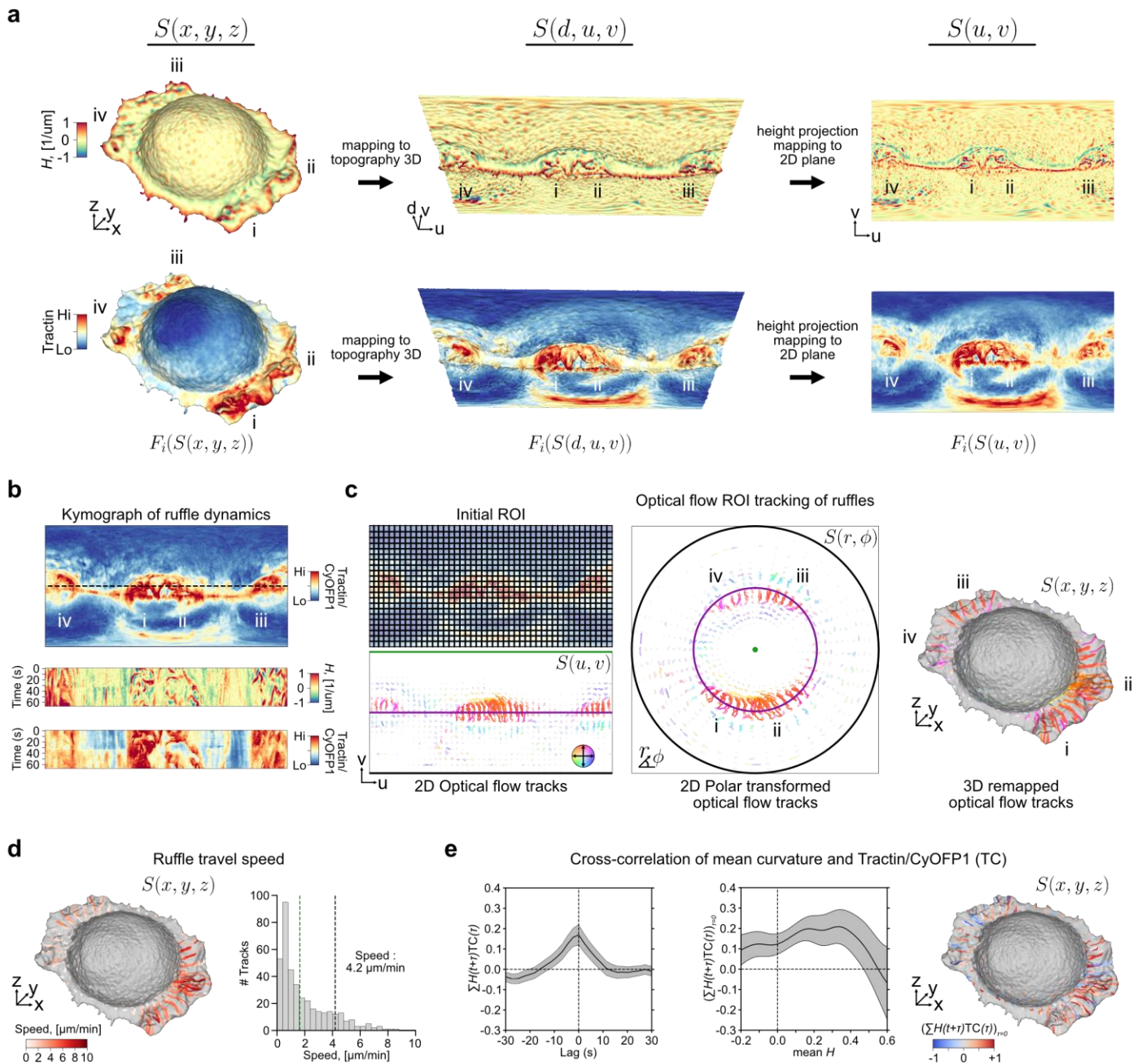
Figure 5. u-Unwrap3D enables tracking and characterization of morphological and molecular dynamics. a) Unwrapping of a ruffling SU.86.86 pancreatic adenocarcinoma cell visualized in Cartesian 3D (left), topographic 3D (middle) and unwrapped 2D plane (right) representations for the first frame of a timelapse 3D image sequence sampled every 2.27s for 30 frames. Top row: mean curvature; bottom row: Tractin-mEmerald intensity sampled 1 μm from the cell surface. Labels i-iv indicate corresponding landmarks in all three representations. **b)** Cross-section (dashed black line, top) to generate kymographs (bottom) of mean curvature and Tractin-mEmerald intensity normalized to myristolated CyOFP1 as a diffuse volumetric marker. **c)** Optical flow tracking on equipartitioned regions of interest (ROI) in the (u,v)-plane of ruffles based on the Tractin/CyOFP1 (TC) ratiometric intensity(left). The resultant optical flow ROI tracks are colored by the mean track direction. Color saturation indicates mean track speed. ROI tracks remapped to 2D polar (r, ϕ) view (middle) and to Cartesian 3D (x, y, z) surface representation overlaid on the first time point (right). The polar transform maps the green (top), purple (middle) and black (bottom) horizontal line in the (u, v)-plane to the central green point, purple and black rings in the (r, ϕ)-view, respectively. **d)** Mean temporal planar travel speed of the ruffle-associated ROI tracks from c) plotted onto the Cartesian 3D surface representation of the first time point (left) and histogram (right). We infer a mean ruffle travel speed of 4.2 μm/min corresponding to the faster of the two histogram populations (black vertical dashed lines) using 3-class Otsu thresholding (see Methods). **e)** Cross-correlation curve (mean ± 95% confidence interval) between mean curvature and TC timeseries per ROI track (left). Lag 0 cross-correlation of mean curvature and TC as

322   a function of mean curvature, H (middle); ROI tracks color-coded by cross-correlation magnitude plotted onto
323   the Cartesian 3D surface representation of the first time point (right).

324

325

a Surface distorts under deformation

Input Surface

$S(x, y, z)$

Reference Surface from conformalized curvature flow

$S_{ref}(x, y, z)$

H, [1/um]

b Two types of deformation distortions

'Conformal' deformation: preserves aspect ratio

Error I, conformal error measures aspect ratio distortion
minimum error = 1

'Equiareal' deformation: preserves area ratio

Error II, area distortion error measures ratio of final to initial area
minimum error = 1

Conformal error

$\ln(A_1/A_0)$

'isometric' = 'conformal' + 'area-preserving'
'quasi-' = errors are finite and bounded

c Conformalized mean curvature flow distortions

Stop Iteration

Conformal error

A_1/A_0

Iteration Number

d Conformal spherical map distortions

Reference surface   Spherical map   Conformal error $\ln(A_1/A_0)$

$S_{ref}(x, y, z)$ $S^2_Q(x, y, z)$

H, [1/um]

mean conformal error: 1.005   mean A_1/A_0 error: 0.627

e Area distortion relaxation distortions

Stop Iteration

$S^2_\Omega(x, y, z)$

$S^2_Q(x, y, z)$

Conformal error

A_1/A_0

Iteration Number

Errors at stop

Conformal error

mean conformal error: 1.495

$\ln(A_1/A_0)$

mean A_1/A_0 error: 1.000

g Surface to uv distortion

Conformal error $\ln(A_1/A_0)$

Reference Surface → uv
$S_{ref}(x, y, z) \rightarrow S(u, v)$

mean conformal error: 1.815   mean A_1/A_0 error: 1.000

Input Surface → uv
$S(x, y, z) \rightarrow S(u, v)$

mean conformal error: 2.169   mean A_1/A_0 error: 1.000

f Optimising (u,v) unwrapping axis

Let: point, $P_i = (x_i, y_i, z_i)$ on sphere
w_i = associated weight of point, P_i e.g. curvature

Find:
optimal N-S axis, to visualise w_i on (u,v) unwrapping

Solution:
smallest eigenvector of matrix $A = (WP)(WP)^T$

no rotation

optimized rotated

$S^2_\Omega(x, y, z)$

quasi-equiareal

quasi-equiareal

h

$S(x, y, z)$

SA : 708.87 um²

Reconstructed input 3D surfaces under different spherical parametrization

Input surface to sphere
$S^2(x, y, z) \rightarrow S(x, y, z)$

CD : 0.069 µm
SW_1 : 0.222 µm
ΔA : +43.85 µm²
SA : 752.72 µm²

Input surface to quasi-conformal sphere
$S^2_Q(x, y, z) \rightarrow S(x, y, z)$

CD : 0.071 µm
SW_1 : 0.037 µm
ΔA : -2.83 µm²
SA : 706.04 µm²

CD = Chamfer distance
SW_1 = Sliced 1-Wasserstein distance

Input to reference surface to equiareal sphere
$S^2_\Omega(x, y, z) \rightarrow S_{ref}(x, y, z) \rightarrow S(x, y, z)$

CD : 0.065 µm
SW_1 : 0.031 µm
ΔA : -3.32 µm²
SA : 705.55 µm²

ΔA = Difference in surface area from
SA = Total surface area

Input surface to equiareal sphere
$S^2_\Omega(x, y, z) \rightarrow S(x, y, z)$

CD : 0.059 µm
SW_1 : 0.024 µm
ΔA : +0.77 µm²
SA : 709.64 µm²

326

Extended Figure 1. Measuring and optimizing mesh distortion under surface deformation. a) Any deformation of a closed 3D surface mesh (top) such as by conformalized mean curvature flow (cMCF) (bottom) distorts local geometrical distances and areas as illustrated by zoom-ins. **b)** Illustration of the two types of metric distortion incurred by mesh deformation; conformal and equiareal. In general, lower conformal error is at the expense of equiareal and vice versa. **c)** Plot of the conformal error (black dotted line, black left y-axis) and area ratio (red dotted line, red right y-axis) for each iteration of cMCF (Step 1, Fig. 1b) for the example mesh in a) and Fig. 1 with stop iteration indicated by a black vertical dashed line. **d)** Rendering of the conformal error and area ratio error at each triangle face for quasi-conformal spherical parametrization of the smooth shape to the sphere (Step 2, Fig. 1b). **e)** Plot of the conformal error (black dotted line, black left y-axis) and area ratio (red dotted line, red right y-axis) for each iteration of the spherical area distortion relaxation with stop iteration indicated by a black vertical dashed line (Step 3, Fig. 1b), (left). Rendering of the conformal and area ratio error of individual triangle faces at the stop iteration, (right). **f)** Illustration of not optimising (upper row) and optimising the unwrapping north-south axis using weighted principal component analysis to maximally display protrusive surface features with minimal distortion using the absolute value of mean curvature of the smooth 3D shape as weights, w (lower row). **g)** Comparison of the per pixel conformal error (left column) and area distortion (right column) of the 2D (u, v) unwrapping of the cMCF smooth shape, $S_{\mathrm{ref}}(x, y, z)$ (upper row) or direct unwrapping of the input shape, $S(x, y, z)$ (lower row). **h)** Quantitative assessment of four different options of 2D (u, v) unwrapping an input surface, $S(x, y, z)$ via different spherical parameterizations, $S^2(x, y, z)$ by measuring the difference between the Cartesian 3D reconstructed mesh from $S(u, v)$ and $S(x, y, z)$. CD = Chamfer distance, SW_1 = sliced 1-Wasserstein distance between vertices of the input and reconstructed mesh. SA = the total surface. ΔA = difference in total surface area between the input and reconstructed mesh. Qualitative assessment by uv-remapping the chessboard pattern and blending with the mean curvature, H colors.

a

Example of higher genus inducing errors in the input surface meshes

(genus-7)
#V: 163,646
#F: 327,316

Lamellipodia

(genus-2694)
#V: 380,922
#F: 772,900

Filopodia

b

Ushape3D segmented input surface, $S(x, y, z)$ mesh statistics

Protrusion Type	#Vertex (Input) $S(x,y,z)$	#Face (Input) $S(x,y,z)$	#Watertight (Input) $S(x,y,z)$	Genus Range (Input) $S(x,y,z)$	#Genus-0 (Input) $S(x,y,z)$	Face quality (Input) $S(x,y,z)$	# input meshes with a $S_0^2(x,y,z)$	# input meshes with a $S(d,u,v)$
Blebs (n=19)	121.2±58.9k	242.5±118.4k	11 (58%)	0-577	4 (21%)	0.633±0.002	19 (100%)	19 (100%)
Lamellipodia (n=38)	161.1±53.4k	280.2±110.9k	25 (66%)	0-38.5	2 (5%)	0.867±0.003	36 (95%)	36 (95%)
Filopodia (n=9)	357.1±96.4k	719.7±196.8k	0 (0%)	475-4958	0 (0%)	0.663±0.004	8 (89%)	8 (89%)

c

Example of an input mesh from which a topography mesh could not be automatically generated

* cMCF automatic stop iteration # = 11

Iteration # = 0   Iteration # = 5   Iteration # = 11 *   Iteration # = 21   Iteration # = 33

Conformalized mean curvature (cMCF) iterations

d

cMCF and remeshed reference surface, $S_{\mathrm{ref}}(x, y, z)$ mesh statistics

Protrusion Type	conformalized Mean Curvature Flow (cMCF)				Remeshed reference shape properties, $S_{\mathrm{ref}}(x,y,z)$				
	#cMCF iterations	Conformal Error	Median Area Distortion	Mean Area Distortion	#Vertex (Remesh)	#Face (Remesh)	#Watertight (Remesh)	#Genus-0 (Remesh)	Face Quality (Remesh)
Blebs (n=19)	4.0±4.5	1.103±5.880	1.05±0.07	$5.5 \times 10^{-12} \pm 0.13$	101.5±31.2k	203.0±62.4k	19 (100%)	19 (100%)	0.880±0.009
Lamellipodia (n=38)	9.5±4.0	1.458±0.517	0.75±0.21	$3.25 \times 10^{-10} \pm 7.25 \times 10^{-8}$	85.5±125.8k	171.1±251.5k	35 ^ (89%)	35 ^ (89%)	0.885±0.011
Filopodia (n=9)	4.0±4.0	inf *	0.40±0.37	0±0	38.4±17.2k	76.8±34.3k	8 (89%)	8 (89%)	0.825±0.007

* Thin regions part of the bridge part of handles (see panel a) were shrunk to singular thin structures under cMCF, terminating flow early
^ Expected 36 meshes based on the number of topographic meshes but 1 mesh tested not watertight and not genus-0

351

352 **Extended Figure 2. Assessment of the general applicability u-Unwrap3D. a)** Typical examples of higher
353 genus inducing 'hole' and 'handle' errors in 3D meshes obtained by marching cubes meshing of binary
354 segmentations from volumetric lightsheet microscopy images for two morphological motifs and two cell types;
355 a dendritic cell with lamellipodia (left) and a HBEC cell with filopodia (right). **b)** Table summary of the statistics
356 of the total 66 input surface meshes across morphological motif types to u-Unwrap3D (columns 1-6), of which
357 for a total 63 input meshes (>95%) an equiareal spherical parametrization and topographic meshes were
358 successfully computed (columns 7-8, last two columns). In comparison only 6 input meshes (9%) were genus-
359 0. An example of a failed mesh is given in c). **c)** Example of u-Unwrap3D failure when the conformalized
360 mean curvature flow (cMCF) reference shape at the automatic stop iteration has holes too large to be made
361 genus-0 after voxelization and morphological hole closing in our current implementation (see Methods). **d)**
362 Table summary of the conformal and area distortion error evaluated at the automatic stop iteration of the
363 cMCF (left half, first 4 columns) and the summary mesh statistics of the remeshed cMCF smooth mesh (right
364 half, last 5 columns). Note the area distortion is given as the surface area fraction ratio with the input surface
365 $S_{\mathrm{ref}}(x, y, z)$ as the denominator and equivalent to $1/\lambda$ used in the area distortion relaxation (see Methods).
366 (see Methods). All tables in b)-g) report numerical values as median±interquartile range. An inf conformal

367   error indicates local breakdown of flow for a mesh. When this occurs, cMCF cannot continue and the
368   automatic stop iteration is the iteration # just prior to breakdown.

a

Quasi-conformal spherical parameterization, $S_Q^2(x, y, z)$ geometric distortion statistics

Protrusion Type	Conformal Error	Area Distortion
Blebs (n=19)	1.002±0.003	0.978±0.037
Lamellipodia (n=36)	1.007±0.005	0.432±0.813
Filopodia (n=8)	1.016±0.013	0.140±0.580

b

Quasi-equiareal spherical parameterization, $S_\Omega^2(x, y, z)$ geometric distortion statistics for different stopping criteria

Protrusion Type	#iterations (equiareal)	Conformal Error (equiareal)	Area Distortion (equiareal)	Face Quality (equiareal)
Blebs (n=19)	0.0±2.0	1.007±0.036	0.985±0.012	0.879±0.135
Lamellipodia (n=36)	23.0±13.0	1.602±0.946	1.000±0.000	0.771±0.149
Filopodia (n=8)	18.5±7.5	1.690±0.918	1.000±0.001	0.736±0.150

(i) Equiareal parameterization

Protrusion Type	#iterations (MIPs)	Conformal Error (MIPs)	Area Distortion (MIPs)	Face Quality (MIPs)
Blebs (n=19)	0±0	1.002±0.003	0.978±0.037	0.880±0.009
Lamellipodia (n=36)	0±0	1.007±0.005	0.432±0.813	0.885±0.010
Filopodia (n=8)	0±0	1.016±0.013	0.140±0.580	0.825±0.007

(ii) Most isometric parameterization (MIP)

Protrusion Type	#iterations (Q+logλ)	Conformal Error (Q+logλ)	Area Distortion (Q+logλ)	Face Quality (Q+logλ)
Blebs (n=19)	0.0±0.0	1.002±0.005	0.978±0.029	0.880±0.076
Lamellipodia (n=36)	7.5±14.8	1.254±0.594	0.866±0.112	0.846±0.095
Filopodia (n=8)	8.5±6.8	1.365±0.521	0.896±0.107	0.791±0.084

(iii) Conformal + equiareal parameterization

Protrusion Type	#iterations (area-preserve MIPs)	Conformal Error (area-preserve MIPs)	Area Distortion (area-preserve MIPs)	Face Quality (area-preserve MIPs)
Blebs (n=19)	4.0±3.5	1.073±0.056	0.997±0.011	0.877±0.130
Lamellipodia (n=36)	16.5±14.0	1.452±0.670	0.979±0.033	0.800±0.116
Filopodia (n=8)	12.5±4.5	1.550±0.715	0.980±0.028	0.759±0.122

(iv) Area-preserving most isometric parameterization(MIP)

c

Topographic 3D surface, $S(d, u, v)$ mesh statistics

Protrusion Type	#Vertex (Topographic) $S(d,u,v)$	#Face (Topographic) $S(d,u,v)$	Genus Range (Topographic) $S(d,u,v)$	#Genus-0 (Topographic) $S(d,u,v)$	Face Quality (Topographic) $S(d,u,v)$
Blebs (n=19)	389.3±23.9k	776.2±47.8k	0-10.5	17(89%)	0.829±0.001
Lamellipodia (n=36)	535.0±138.2k	1067.6±276.4k	0-4.5	13(36%)	0.831±0.002
Filopodia (n=8)	201.6±21.8k	401.7±43.6k	0-1.5	3(38%)	0.873±0.003

d

Geometric error between Cartesian 3D remapped Topographic, $S_{topo}(x, y, z)$ and input, $S(x, y, z)$ surface mesh

Protrusion Type	Chamfer Distance, CD (voxel)	Sliced 1-Wasserstein, SW_1 (voxel)	% Difference in surface area, ΔA	% Difference in volume, ΔV
Blebs (n=19)	2.79±6.72	4.28±7.24	-11.6±43.9% *	+4.2±0.6%
Lamellipodia (n=36)	1.77±0.16	0.93±0.33	+1.2±5.4%	+7.9±1.4%
Filopodia (n=8)	10.33±2.78	18.45±5.35	-55.3±13.0% *	+3.4±1.2%

* inflated error due to erroneous meshing of internal volume structures in input mesh

e

Topographic 3D $S(d, u, v)$ → remapped Cartesian 3D $S_{topo}(x, y, z)$ | input Cartesian 3D $S(x, y, z)$ | cMCF Reference $S_{ref}(x, y, z)$

Bleb
CD : 10.78 voxel
SW_1 : 17.20 voxel
ΔA : -56.6%
ΔV : +4.4%

Lamellipodia
CD : 1.79 voxel
SW_1 : 0.77 voxel
ΔA : -0.5%
ΔV : +8.9%

Filopodia
CD : 9.05 voxel
SW_1 : 25.93 voxel
ΔA : -57.4%
ΔV : +2.7%

Lamellipodia
CD : 1.79 voxel
SW_1 : 1.21 voxel
ΔA : 0.8%
ΔA : 8.0%

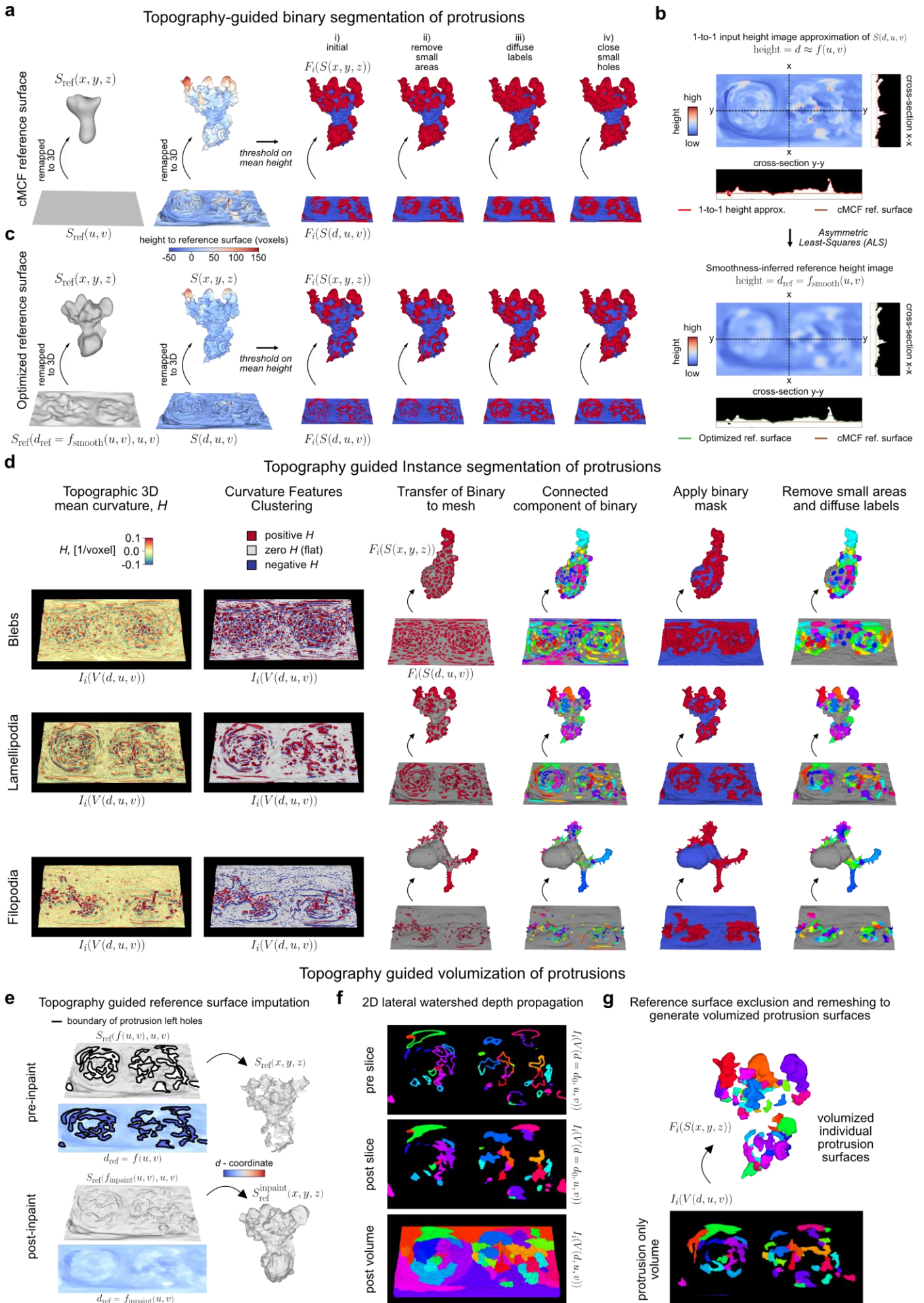► well recovered features   ► recovered features   ► not recovered features

Extended Figure 3. Quantitative performance assessment of the geometric deformation steps in u-Unwrap3D. a) Table summary of the conformal and area distortion error of quasi-conformal spherical parametrization of the cMCF reference surface, $S_{ref}(x, y, z) \rightarrow S_Q^2(x, y, z)$, (Step 2 Fig. 1b). The target and optimal minimum conformal error is 1.0 and is achieved. **b)** Table summary of the number of iterations,

369
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374 conformal and area distortion error and mesh quality for four different stopping criteria (labelled i-iv, see
375 Methods) for area-distortion relaxation of $S_Q^2(x, y, z) \rightarrow S_\Omega^2(x, y, z)$. The target and optimal minimum area
376 distortion is 1.0. The implemented spherical area-distortion relaxation scheme in this paper achieves the
377 optimal area distortion, an equiareal parametrization in $t_\Omega <$ a maximum allowed 50 iterations by minimising
378 directly the area-distortion factor, λ (equiareal) criterion (Methods) (top left, i). The scheme further allows
379 relaxations between conformal and equiareal parametrizations as demonstrated by three additional stopping
380 criteria: with no relaxation, (i.e. iteration 0) the parameterization is conformal and the most isometric
381 parametrization (MIPS) (top right, ii); for iteration numbers $t \approx \frac{1}{2} t_\Omega$, the parameterization minimises jointly
382 the combined conformal and area distortion as measured by $Q + \log \lambda$, the sum of the quasi-conformal
383 error, Q and the natural logarithm of the area distortion factor, λ (bottom left, iii); and for iteration numbers
384 $t \lesssim t_\Omega$, the parameterization is the area-preserving MIPS (bottom right, iv). **c)** Table summary of the statistics
385 for computed topographic meshes, $S(d, u, v)$ using a 1024×512 pixel (u, v) grid for the subset of $n =63$
386 meshes with successful equiareal spherical parametrizations, $S_\Omega^2(x, y, z)$ (Extended Fig. 2b). **d)** Table
387 summary of the quantitative measurement of geometric error between the Cartesian 3D remapping,
388 $S_{\text{topo}}(x, y, z)$ of the topographic mesh, $S(d, u, v)$ and the original input mesh $S(x, y, z)$ for four metrics; (CD)
389 chamfer distance (1st column), (SW_1) sliced 1-Wasserstein (2nd column), ΔA, the percentage difference in total
390 surface area (3rd column) and ΔV, the percentage difference in total volume (4th column). For a perfect
391 reconstruction, all measures should be 0. Units are given as voxels due to heterogeneous pixel resolution
392 amongst input meshes. A large ΔA, but small ΔV for blebs and filopodia were due to a subset of non-
393 watertight input meshes found to have erroneously meshed what should be the internal cell volume. These
394 meshes were typically characterised by very high-genus (>50) (Extended Fig. 2c). Note in a)-d) the area
395 distortion is given as the surface area fraction ratio with the input surface $S_{\text{ref}}(x, y, z)$ as the denominator and
396 equivalent to $1/\lambda$ used in the area distortion relaxation (see Methods). All tables in a)-d) report numerical
397 values as median $\pm$ interquartile range. **e)** Quantitative and qualitative comparison of the Cartesian 3D
398 remapping, $S_{\text{topo}}(x, y, z)$ of the topographic mesh, $S(d, u, v)$ (left) and the original input mesh $S(x, y, z)$
399 (middle) for 4 cell examples with different morphological motifs from d) in relation to the cMCF reference
400 surface, $S_{\text{ref}}(x, y, z)$ (right). Box shows a zoom-in of the local surface region for each example. Black triangles
401 highlight exemplar salient surface that are well captured but may be slightly smoothened and blurred in
402 $S_{\text{topo}}(x, y, z)$ due to being underrepresented surface regions in $S(d, u, v)$, being distant from $S_{\text{ref}}(x, y, z)$.
403 Green triangles highlight exemplar salient filopodia captured in $S_{\text{topo}}(x, y, z)$. Red triangles highlight exemplar
404 salient filopodia not or poorly captured in $S_{\text{topo}}(x, y, z)$, due to being in a region of dense filopodia and is
405 distant from $S_{\text{ref}}(x, y, z)$.

406

407

a Topography-guided binary segmentation of protrusions

b 1-to-1 input height image approximation of $S(d,u,v)$
$$\text{height} = d \approx f(u,v)$$

Asymmetric Least-Squares (ALS)

Smoothness-inferred reference height image
$$\text{height} = d_{\text{ref}} = f_{\text{smooth}}(u,v)$$

c Optimized reference surface

d Topography guided Instance segmentation of protrusions

Topographic 3D mean curvature, H | Curvature Features Clustering | Transfer of Binary to mesh | Connected component of binary | Apply binary mask | Remove small areas and diffuse labels

Blebs

Lamellipodia

Filopodia

Topography guided volumization of protrusions

e Topography guided reference surface imputation

f 2D lateral watershed depth propagation

g Reference surface exclusion and remeshing to generate volumized protrusion surfaces

408

Extended Figure 4. Overview of an unsupervised pipeline to segment complex surface morphologies guided by topographic 3D representations.

a) Overview of the four key steps (i-iv) to binary segment protrusions by thresholding on the mean height measured relative to the cMCF reference surface. **b)** Learning an optimal reference surface for segmenting surface protrusions using asymmetric least mean squares (ALS) (Methods). The topographic surface, $S(d, u, v)$ is approximated by a surface, $S(d = f(u, v), u, v)$ that is in 1-to-1 correspondence to every (u, v) pixel and can be represented as a height image using ray-propagation (top, see Methods). The height function, f is depicted additionally in cross-section cuts x-x and y-y by a red line. A flat brown line in cross-section depicts the cMCF reference, $S_{\mathrm{ref}}(d, u, v)$. ALS with smoothness regularization is applied to $d = f(u, v)$ to derive an optimal smooth reference surface with height, $d = f_{\mathrm{smooth}}(u, v)$ (bottom). The height function, f_{smooth} are depicted additionally in cross-section cuts x-x and y-y by a green line. **c)** Binary segmented protrusions by thresholding on the mean height measured relative to the ALS-derived reference surface from b). **d)** Overview of the sequential steps, left-to-right to segment individual protrusions by binarization and connected components analysis of topographic volume signals, $I_i(V(d, u, v))$. The steps are illustrated for 3 different cell types and 3 different surface motifs; MV3 melanoma cell with blebs (top row), dendritic cell with lamellipodia (middle row) and HBEC cell with filopodia (bottom row). Initial binarization uses 3-class k-means clustering (blebs and filopodia) and 3-class Gaussian mixture model clustering (lamellipodia) of volumetric mean curvature to identify all positive curvature regions. **e)** The hole-ridden ($d = 0$, black outline, and dark blue colored) reference surface, $S_{\mathrm{ref}}(d_{\mathrm{ref}} = f(u, v), u, v)$ (left) and corresponding remapped Cartesian 3D surface (right) after removal of all protrusion mesh faces (top). The inpainted topographic 3D reference surface, $S_{\mathrm{ref}}(d_{\mathrm{ref}} = f_{\mathrm{inpaint}}(u, v), u, v)$ (left) and corresponding remapped Cartesian 3D surface (right) after image inpainting the missing d coordinates (bottom). **f)** Marker-seeded 2D watershed to laterally propagate surface segmentation labels into the topographic volume shown pre- (top) and post- (middle) for a single given slice at depth, $d = d_0$. The resulting labelled topographic volume after propagating all surface protrusion labels fully from the top to the bottom (bottom). Unique colors denote unique regions with the same surface label. **g)** The protrusion-only topographic volume labels (bottom) and resultant composition of individual volumized protrusion meshes (top) after using the inpainted basal surface from e) to mask out all non-protrusion voxels in f) and meshing. Individual protrusions are uniquely colored.
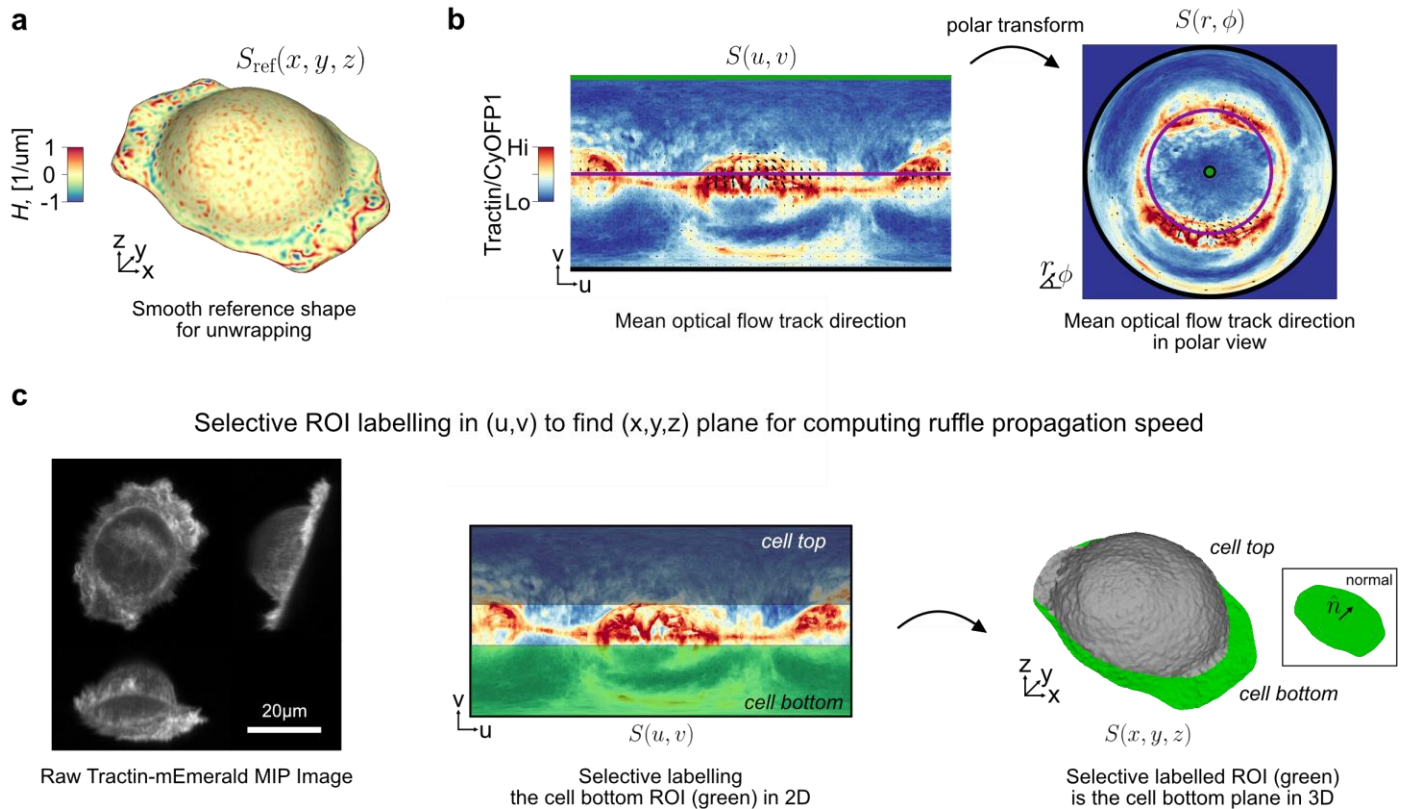
Extended Figure 5. u-Unwrap3D enabled measurement of ruffle in-plane travel speed. a) Cortical reference shape, $S_{\text{ref}}(x, y, z)$ found by applying conformalized mean curvature flow to the first timepoint of the ruffling SU.86.86 cell in Fig. 5a and used to unwrap all timepoints into a common static (d, u, v) space. **b)** The mean track velocity of the optical flow region-of-interest (ROI) tracks, plotted as black arrows at the initial track coordinate overlaid on the unwrapped Tractin-mEmerald/CyOFP1 intensity image of Fig. 5b (left) and its corresponding polar transformed equivalent (right). The polar transform maps the green (top), purple (middle) and black (bottom) horizontal line on the left to the central green point, second purple and third black rings in the polar image. **c)** Orthogonal cross-section maximum intensity projection (MIP) image of the raw Tractin-mEmerald intensity channel showing the tilted lightsheet acquisition (left). Selective ROI isolation of the top and bottom surface of the cell by grey and green bounding box selection in unwrapped view (middle) and visualized in 3D with grey and green surfaces respectively (right). The normal vector, $\hat{n}$ describing the best fit plane through only the cell bottom vertices found by principal components analysis (inset rectangle).

Supplementary Videos

Supplementary Video 1. Overview of the six key steps of u-Unwrap3D.

Supplementary Video 2. Application of u-Unwrap3D to directly unwrap a genus-0 cell surface mesh in conjunction with the spatial activation pattern of PI3K signaling products.

Supplementary Video 3. Application of u-Unwrap3D to an MV3 melanoma cell with bleb surface motifs segmented by the u-shape3D software. Each bleb is labelled with a random color to demonstrate the local surface mappings and their distortions.

Supplementary Video 4. Application of u-Unwrap3D to a dendritic cell with lamellipodia surface motifs segmented by the u-shape3D software. Each lamellipodium is labelled with a random color to demonstrate the local surface mappings and their distortions.

Supplementary Video 5. Application of u-Unwrap3D to an HBEC cell with filopodia surface motifs segmented by the u-shape3D software. Each filopodium is labelled with a random color to demonstrate the local surface mappings and their distortions.

Supplementary Video 6. Application of topographic conformalized mean curvature flow to directly map the topographic surface $S(d, u, v)$ of a dendritic cell with segmented lamellipodia surface motifs to the 2D plane, $S(u, v)$ for two different time steps, $\delta = 100$ and $\delta = 5000$. The smaller δ enables gradual relaxation and the ability to sample and use intermediate shapes during the flow. However smooth low curvature folds remain such that we do not fully converge to the plane even if continued to 100 iterations. For direct mapping to the plane we always use the large δ to ensure convergence within 50 iterations.

Supplementary Video 7. Application of u-Unwrap3D to enable segmentation and tracking of blebs on a MV3 melanoma cell in topographic representation. View 1: Projections of cell surface, mean curvature and normalized SEPT6-GFP into topographic surface and (u, v) unwrapped reference surface representations. Individual blebs are segmented by thresholding in the topographic surface representation. Leveraging the bijectivity of u-Unwrap3D mappings bleb labels are projected back to the 3D surface. View 2: The 2D segmented blebs are tracked and trajectories projected back to the original 3D surface. The timelapse volumes were acquired every 1.21s for 200 frames. Scalebar: 10μm.

Supplementary Video 8. Application of u-Unwrap3D to track the surface ruffling and actin flows of a SU.86.86 pancreatic adenocarcinoma. View 1: Projections of cell surface, mean curvature and Tractin-mEmerald into topographic surface and (u, v) unwrapped reference surface representations. View 2: Regional ruffling and actin flows are tracked in 2D with optical flow and trajectories projected into a 2D polar representation and back to the original 3D surface. View 3: Select measurement of instantaneous ruffle and actin flow speeds and cross-correlation of actin and curvature within the lamella and lamellipodia taking advantage of the unwrapped (u, v) representation is projected back to the original 3D surface. The timelapse volumes were acquired every 2.27s for 30 frames. Scalebar: 20μm.

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