Continuum mechanics modeling of *Drosophila* mesoderm invagination based on spherical harmonics accounts for long-range tissue remodeling

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Abstract

Mechanics plays a key role in the development of higher organisms. For example, during fruit-fly gastrulation, local forces generated by the acto-myosin meshwork in the region of the future mesoderm lead to formation of a ventral tissue fold. The process is accompanied by substantial changes in cell shape and long-range cell movements whose origin is not understood.

It has proven difficult to model the link between local forces, generated at the subcellular level, and global tissue deformation. Here, we adopt an approach first developed for lipid bilayers and cell membranes, in which we model force-generation at the cytoskeletal level as resulting in local changes in preferred tissue curvature. The continuum mechanics problem can thus be completely formulated in terms of tissue strains, which is desirable since mechanical forces themselves are often unknown. The solution then yields global morphogenetic predictions that accommodate the tendency towards this local preferred curvature. Computer simulations, especially in three dimensions, face the additional challenge of high complexity of shapes, gene expression patterns, and mechanical constraints. Our computational framework, which we call SPHARM-MECH, extends a three-dimensional spherical harmonics parameterization known as SPHARM to address this challenge.

Using SPHARM-MECH in combination with whole-embryo light-sheet-based live imaging, we study mesoderm invagination in the fruit-fly embryo. Our analysis reveals a striking correlation between calculated and observed tissue movements, predicts the observed cell shape anisotropy on the ventral side of the embryo, and suggests an active mechanical role of the mesoderm invagination process in supporting the first phase of germ-band extension.

Abbreviations

MP: mesoderm primordium; VFI: ventral furrow invagination, CF: cephalic furrow.

Introduction

In the course of embryonic development, the embryo undergoes fundamental changes in shape, which typically involve long range tissue reorganization and epithelial folding. At the cellular and molecular levels, these morphogenetic processes are driven by reorganization of the cytoskeleton, which leads to the generation of forces[1, 2, 3, 4]. This activity is orchestrated at the whole-embryo level through the differential expression of genes, which control the spatial and temporal patterns of force generation. In addition, mechanical constraints determine and limit the morphological configurations available to the developing organism, and the forces generated in turn influence gene expression itself[5, 6, 7]. It is thought that forces are transmitted over long ranges through the mechanical properties of the tissue. However, the details of how forces exercise this long-range effect can only be understood by combining experimental data and detailed computational mechanics models on a case-by-case basis. With advances in microscopy technology, detailed live imaging data of whole-embryo morphogenesis have become available[8, 9] (**Fig. 1a** and **Supplementary Videos 1** and **2**). Moreover, sophisticated large-scale approaches to imaging and computational image analysis have produced near-comprehensive quantitative gene expression distribution maps for the early embryo[10, 11] (**Fig. 1b**). With the availability of such global information, we aim at predicting observed tissue movements and cell shape changes that result from local force generation, by considering whole-embryo tissue mechanics.

There have been a number of efforts to model morphogenesis on the computer[12]; for example: in the fruit fly[13, 14, 15], the sea urchin[16, 17], and the chick embryo[18, 19]. However, the accurate general three-dimensional modeling of embryogenesis is a difficult task. A major challenge is the requirement of a realistic mathematical representation that is able to express the inherent three-dimensionality of the full embryogenesis problem accurately and efficiently. This is essential to capture the long-range character of morphogenesis and the fast morphological changes that often occur simultaneously in the whole embryo. The representation must accommodate the complex intermediate and emerging morphologies, gene product distributions and mechanically relevant gene product interactions as well as the constitutive tissue behavior. A second challenge is to link the force generating mechanisms, which occur at the subcellular level through cytoskeletal dynamics, to a continuum mechanics formulation (which is necessary for performing whole-embryo simulations in realistic computation times). This link should provide a level of

abstraction that is able to approximate the activities of various cytoskeleton-level mechanisms, and yet communicates their effects on a macroscopic scale in a manner compatible with standard mechanics treatments.

To address the above challenges, we developed an open-source computational biomechanics framework which we call SPHARM-MECH (**Fig. 1**). Its internal data representation is based on the spherical harmonics basis functions, which comprise a powerful Fourier basis for approximating functions on the sphere (**Supplementary Fig. 1** and **Supplementary Note 1**). The spherical harmonics have been used to represent, register and compare complicated morphologies of simply-connected objects through an approach known as SPHARM[20, 21, 22]. SPHARM-MECH data representation generalizes the SPHARM approach. It concisely encodes, in addition to morphology, gene expression distributions to facilitate the data-driven identification of regions of local gene-product activity (**Fig. 1a-d** and **Methods**). Its fidelity to experimental data is limited only by the spatial and temporal resolution of the recording device and noise. Harmonic functions of higher order gradually provide more detail, as expected from a converging Fourier series (**Supplementary Fig. 2**). Importantly, gene expression patterns are naturally mapped onto the morphologies independent of their source in a straight-forward manner (**Fig. 1b-d, Supplementary Fig. 3a-g** and **Supplementary Note 5**). Using these maps, regions of local mechanical activity are generated (**Fig. 2a** and **Supplementary Fig. 3h**).

Given gene activity maps, a starting morphology (**Fig. 1d**), and material properties **(Fig. 1e**), SPHARM-MECH calculates a configuration-dependent strain energy for the whole tissue (**Fig. 1f** and **Methods**). We propose that the driving force for invagination manifests itself mechanically as a departure of the preferred tissue curvature from its current curvature at the locations of gene product activity. This can occur via a number of different molecular and cellular mechanisms. Examples include the contraction of an acto-myosin meshwork driving the ventral furrow invagination (VFI) (**Fig. 1h**) via apical cell constriction[23], or the repositioning of adherence junctions responsible for formation of a dorsal fold[24], both observed in the fruit-fly embryo. Especially in the fruit-fly system, the idea of change in local preferred curvature is justified. The egg shell and vittelline membrane act as mechanical constraints. They will only allow a frustrated high-energy configuration to be mechanically relieved by buckling the tissue towards the interior. The preferred curvature concept provides the necessary link between details

of the biological force-generating mechanism and the continuum tissue mechanics formulation. As mentioned above, morphological changes occur in order to relieve the strained (high energy) tissue configuration (**Fig. 1f**). SPHARM-MECH predicts the morphology that minimizes this strain energy (**Fig. 1g**) by using standard methods of numerical optimization (**Methods**).

In this work, we apply SPHARM-MECH to model VFI formation in *Drosophila*. We show that the forces generated by the isotropic contraction of the acto-myosin meshwork local to the future mesoderm, are responsible for the observed tissue anisotropy on the ventral side of the embryo. We also show that these forces are sufficient to support tissue movements of the posterior pole in the dorsal direction, thereby supporting the first phase of germ-band extension, and that this effect is directly related to the geometry of the egg.

Results and Discussion

We use a combined experimental and theoretical approach to investigate gastrulation mechanics. Time-lapse, three-dimensional image data of entire *Drosophila* embryos are recorded during gastrulation, using simultaneous multiview light-sheet microscopy (SiMView microscopy)[25]. Through image processing (**Methods**) this provides data of cell nuclei positions and cell shape dynamics throughout this process (**Supplementary Videos 1** and **2**). This data is used to obtain the three-dimensional starting morphology which drives the model, as well as the time evolution of the embryo shape outline which allows us to quantitatively assess the predictive power of the SPHARM-MECH modeling framework.

The main idea of SPHARM-MECH is to efficiently and accurately predict morphogenetic changes at the whole-embryo scale based on continuum shell mechanics strain energy calculations. This computational framework expresses the shape outline (**Fig. 1a**) and gene expression patterns (**Fig. 1b**) in terms of a small number of shape descriptors using the spherical harmonics basis functions (**Fig. 1d**), calculates a configurational strain energy (**Fig. 1f**), and yields a predicted configuration that minimizes this energy (**Fig. 1g**) using numerical optimization. This solves the mechanical problem purely in terms of strains, which is desirable, because information about stresses is not available, and strains constitute the main observable in

our microscopy experiments. Details about the spatial scheme and strain energy calculation are provided in **Methods** and in **Supplementary Notes 1-6**.

We use SPHARM-MECH to model early VFI formation in *Drosophila melanogaster*; a system where significant advancements in understanding gastrulation[26, 27, 28], the associated gene expression patterns and gene product activity[10, 11] have been made. VFI formation is accompanied by a sequence of cell shape changes that ultimately lead to internalization of the mesoderm[27, 28, 29, 30]. The first change in the shapes of cells forming the VFI is apical constriction, which is driven by acto-myosin-contraction[23, 31]. Apical constriction is local to expression of *twist* and *snail*, both of which are essential for furrow formation and are expressed ventrally along the anterior-posterior axis. This contraction is compatible with our assumption that preferred curvature is induced locally. *huckebein* sets the limits of *snail* and *twist* expression levels at the poles and *dorsal* modulates the relative advancement of cells in their fate (**Supplementary Fig. 3g,h**). We calculate two different acto-myosin contractility maps (**Fig. 2a**), and perform a quasi-static mechanics simulation using each. The starting surface is obtained from the outline of the blastoderm prior to the onset of gastrulation (**Fig. 2b** top). The local preferred curvature remains equal to that of the blastoderm everywhere except in regions of activity as determined by the activity map. Values of local preferred curvature within the activity region are input as multiples (γ) of the image-data-determined curvature about 10 minutes into gastrulation. In those regions, γ is increased in two steps (to 2 and 20, respectively). In each step a full numerical optimization of the strain energy is performed to yield the predicted morphology and strain field.

From previous work using electron microscopy, it is known that a core band of cells about 8-10 cell-width contracts prior to the rest of the MP[27]. Using the expression patterns for *dorsal* and *huckebein* (**Fig. 1b-d, Supplementary Fig. 3g,h** and **Supplementary Note 8**), and the strategy mentioned above, we find that a map corresponding to an eight-cell wide ventral anterior-posterior oriented band (**Fig. 2a)** reproduced the initial stage of VFI successfully (**Fig. 2b,c)** when compared to experimental data obtained with SiMView microscopy (**Supplementary Video 1**) and other imaging modalities (**Supplementary Fig. 4**). This is in contrast to the case when considering the activity of *snail*, *twist* and *huckebein* (**Supplementary Note 8**) to generate a map that is thresholded to cover 1/6 of the blastoderm surface, which is equivalent to the

mesoderm primordium region (MP) (**Fig. 2a** and **Supplementary Fig. 3h**, bottom). When this map is used directly to define the region of contractility, i.e. the full MP region is contracting simultaneously, we find that the simulation does not reproduce the observed deformation even at $\gamma = 100$, but produces a frustrated ventrally slightly flattened morphology that does not invaginate. It is thus conceivable that constriction occurs in an out-ward fashion in which cells are activated as a function of stress induced by neighbors. We obtain the same result above when simulating cephalic and ventral furrow formation simultaneously (**Fig. 2d** and **Supplementary Note 11**).

The true power of three-dimensional simulations at the scale of the whole embryo becomes apparent when forces have long-range effects and induce non-trivial global changes in tissue organization. We perform VFI-only simulations using SPHARM-MECH (as above with $\gamma = 20$). From both starting and final configurations we calculate the predicted tissue velocity field. This corresponds to time points zero to ~10 minutes into gastrulation (**Fig. 3a**). Experimentally observed tissue flows (and velocities) are extracted, by image processing, from *in vivo* whole-embryo data of morphogenesis recorded with SiMView microscopy, and covering the same time period (**Fig. 3b**). Both speeds and flow directions for simulation and experiment are compared in the form of histograms. We find very good qualitative and quantitative correspondence in material velocity fields across the entire embryo, including lateral-anterior, ventro-lateral and posterior regions that exhibit the most striking directed tissue flows (**Fig. 3c,d**). Importantly, we note the dorsal flow of tissue in the posterior pole region. This flow may appear counterintuitive at first because the simulation considered deformation resulting from VFI driving forces only, and these driving forces relate to a spatial location that is rather distant from the affected posterior pole region. The simulation thus suggests that the process that forms the ventral furrow favors a dorsal movement of posterior tissue, independent of mechanisms of germ-band extension as a long-range effect. This result is consistent with recent findings that explicitly propose that cell shape change observed during germ-band extension is a passive response to mechanical forces caused by the invaginating mesoderm[32].

To investigate the physical origin of this differential tissue flow, we take advantage of the conciseness of SPHARM-MECH's data representation. In particular, that the fly syncytial blastoderm morphology can be reasonably approximated by only four numbers, each of which is

responsible for the breaking of a different type of symmetry (**Fig. 4a**). Using this reduced set, we perform a series of VFI simulations for symmetric and asymmetric embryo outline approximations. Six simulations are performed for each shape outline and an angular histogram for the lateral-anterior, ventro-lateral and posterior regions is generated (**Fig. 4b**). In addition to the approximate outlines, we include shapes with both their anterior and posterior features identical (a-p symmetric), but based on the original (experimental) outline (**Fig. 4c**). Finally, an average deviation score, as compared to the experimental tissue flows, is calculated (**Fig. 4d**). In the all cases of a-p symmetric outlines no preferred pole for the dorsal movement was observed when considering the averaged simulations. Shapes that were a-p asymmetric, however, showed a clear preference for dorsal movement of posterior tissue (in all cases, $n = 6$). We conclude that the break of a-p symmetry (non-zero value of the 4th shape parameter in **Fig. 4a**) of the egg, coupled to the positioning and geometry of the VFI-region (in which the driving forces originate), are responsible for the differential tissue flow.

In addition, and related to the results above, the simulated tissue flow pattern qualitatively predicts the anterior-posterior anisotropy of the tissue of the VFI, which has been observed experimentally. Using SiMView recordings of fluorescently membrane-labeled whole-embryos (**Fig. 5a,b**), the data is segmented to obtain three-dimensional cell outlines (**Fig. 5c**). In a cross-section of the embryo (cut through and orthogonal to the middle of the a-p axis), we observe good agreement of changes in cell shapes (experimental) vs. simulated tissue deformation (**Fig. 5c-e**). Anisotropy with respect to the a-p axis is calculated for all segmented cells (experimental) and from the strain field (simulated) corresponding to changes within the first 10 minutes of onset of gastrulation, and mapped to the embryo outline, showing good agreement (**Fig. 5f,g**). It is important to note that, although the outcome of the simulation is anisotropic deformation, the simulation correctly used isotropic contraction of the cytoskeleton, by enforcing an isotropic preferred curvature change. This is in accord with the observed isotropic contraction of the acto-myosin meshwork determined experimentally[33]. In the context of mechanical strain energy, this result can be explained as follows: due to the (isotropic) contractile forces of the actin-myosin meshwork, the epithelial tissue surrounding the mesoderm primordium region is forced to extend towards the forming VFI. If this tissue is at (or near) the poles, then it has to change its curvature significantly (departing from its high preferred curvature). This comes at a high energy cost. It is easier (lower energy) for tissue to be "pulled" from the lateral parts of the embryo towards the

furrow region, because only a relatively small change in curvature is associated with its extension towards the ventral side. This result suggests plasticity in the epithelium that sets in at a time prior to the onset of VFI and in which the egg serves as a scaffold, and indicates a direct mechanical influence the morphology of the egg exerts on fly embryo development.

This work demonstrates whole-embryo mechanics modeling that is able to predict global changes in tissue flows based on local forces, as well as the resulting tissue anisotropy. This was facilitated by a computational biomechanics framework that is data-driven and inherently three-dimensional, accommodates a large range of morphological, gene expression and material property data independent of data source, and unifies its analysis within an accurate tissue mechanics context. This framework is provided as **Supplementary Software 1-4**. **Supplementary Videos 3-6** provide a brief introduction to its user interface. We envision that this approach can be applied to a wide spectrum of developmental biology model systems, and will facilitate the testing of effects of mechanical and genetic perturbation in a biomechanical context.

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Methods

SPHARM-MECH spatial scheme

The embryo morphology, together with the expression patterns for all relevant genes, are represented parametrically in functional form as

$$\vec{S} = \begin{bmatrix} x \\ y \\ z \\ \vdots \\ I_g \\ \vdots \end{bmatrix} = \begin{bmatrix} \sum_{L=0}^{\infty} \sum_{K=-L}^{L} C_{LK}^{x} Y_{LK}(\theta,\phi) \\ \sum_{L=0}^{\infty} \sum_{K=-L}^{L} C_{LK}^{y} Y_{LK}(\theta,\phi) \\ \sum_{L=0}^{\infty} \sum_{K=-L}^{L} C_{LK}^{z} Y_{LK}(\theta,\phi) \\ \vdots \\ \sum_{L=0}^{\infty} \sum_{K=-L}^{L} C_{LK}^{g} Y_{LK}(\theta,\phi) \\ \vdots \end{bmatrix} \tag{1}$$

where θ and ϕ are spherical polar coordinates on an abstract unit sphere, and the $Y_{LK}(\theta,\phi)$ are spherical harmonics functions of order L and degree K (**Supplementary Note 1**). The surface outline (in form of Cartesian coordinates) is encoded in the coefficients C_{LK}^{x}, C_{LK}^{y} and C_{LK}^{z}. Scalar fields I_g, encoded in the coefficients C_{LK}^{g}, represent gene expression pattern intensities, for example: I_{snl}, I_{twi}, I_{hkb} and I_{dfd}, as well as the activity maps that determine location and extent of changes in preferred curvature from that of the blastoderm. Gene expression patterns are calculated based on data from the VirtualEmbryo project[10], and from SiMView microscopy recordings in case of the *dorsal* expression pattern, and are assumed constant over the simulation time-frame spanning about 10 minutes after onset of gastrulation. In principle, SPHARM-MECH is not limited to gene expression or activity maps, but may include other field characteristics, for example position-dependent material properties.

The total number of coefficients for the outline is 3 x $(L_{\max} +1)^2$, where $L_{\max}$ is the maximum order of the series. The infinite sum in Eq. 1 is truncated at $L_{\max} = 36$ in all simulations. This provides sufficient accuracy, even for patterns that are highly localized, such as *ftz* and *eve* (**Fig. 1d**, **Supplementary Fig. 3c**), and for more complex embryo morphologies (**Supplementary Fig. 2**). However, it should be noted that higher values of $L_{\max}$ would be necessary to reach single-cell

resolution throughout the embryo. In addition, symmetry is imposed to decrease the number of shape coefficients by about 40%. Total volume, local mean curvature, shear and stretch deformation are evaluated using numerically stable recursion relations[34]. For details see **Supplementary Notes 2-4**.

SPHARM-MECH combines the following four characteristics: (1) The highly accurate evaluation of the spherical harmonics functions and their derivatives[34] provides reliable deformation gradient tensors and therefore shear and stretch energies, as well as accurate local mean curvatures and therefore accurate bending energies (**Supplementary Notes 2** and **3**), (2) fast evaluation of surface integrals is possible by Gaussian quadrature (**Supplementary Note 4**), (3) the concise shape representation makes it practical to use standard techniques of numerical optimization and the descriptors have physical meaning (**Fig. 4a**), and (4) the structure of the spherical harmonics representation naturally takes advantage of symmetry (**Supplementary Note 7**). The main limitation of SPHARM-MECH is the restriction to shapes that are topologically equivalent to the sphere, i.e. of genus zero.

Finally, it should be noted that the spherical harmonics parameterization is not the only shape description appropriate for this kind of problem. Any complete set of orthogonal eigenfunctions defined on the sphere could in principle be used. However, the four characteristics mentioned above provide the SPHARM-MECH framework with particular computational utility.

Mechanical strain energy

We use a generalized neo-Hookean constitutive model based on the work of L.R.G. Treloar for rubber-like materials[35]. The strain energy density is given by:

$$\Phi = \frac{\mu}{2}(\overline{I_1} - 3) + \frac{K}{2}(J - 1)^2 \qquad (2)$$

where μ is the second Lame constant (shear modulus), K the bulk modulus, $J = \lambda_1\lambda_2\lambda_3$ (determinant of the left Cauchy-Green deformation tensor), and $\overline{I_1}$ (an invariant of the left Cauchy deformation tensor) given by $\overline{I_1} = J^{-2/3}(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)$. The principal stretches λ_i (i = 1, 2, 3) are calculated from the shape coefficients of both the current and undeformed (starting)

surfaces using differential geometry (**Supplementary Notes 3** and **6**). The total contribution of shear and stretch energies is given by:

$$E_{\text{strain}} = h \oint_{S_o} \Phi dA \tag{3}$$

where h is the thickness of the shell (assumed constant) and the integration is performed over the undeformed surface S_o. For information on evaluating Eq. 3 numerically see **Supplementary Note 6**. The energy contribution from bending and twisting is given by:

$$E_{\text{bending}} = \frac{k_b}{4} \oint_S (H - C_o)^2 dA, \quad \text{with} \quad k_b = \frac{Eh^3}{12(1-\nu^2)} \tag{4}$$

where E is the Young's modulus, ν Poisson's ratio, H the local mean curvature of the current configuration (**Supplementary Note 3**), and C_o the preferred local mean curvature induced by gene activity. The total energy of the shell is given by $E_{\text{shape}} = E_{\text{strain}} + E_{\text{bending}}$. Predicted shapes were those of minimum E_{shape}, as found by using the constrained nonlinear function optimization SubPlex algorithm of the NLopt C++ library[36, 37].

Model parameters and assumptions

Our primary model assumptions are:

(a) Furrow forming forces affect the geometry through imposing a preferred curvature that is different from the preferred curvature before gene product activity starts (e.g. contraction of cytoskeletal elements).
(b) Regions of constriction cause local stiffening (larger bending resistance) relative to the passive surrounding tissue, i.e. a differential bending modulus of the active region relative to surrounding tissue. In chick embryo the bending stiffness within the region of initiating cells is about twice as high as the surrounding tissue[18].
(c) All tissue deforms passively according to a hyperelastic material model (see above).
(d) The yolk is a fluid that preserves the internal volume enclosed by the embryo during deformation and its viscosity is ignored (constant volume is enforced).
(e) The vitteline membrane forms a hard constraint surrounding the embryo tissue.
(f) Thin shell mechanics are sufficient to approximate the deforming surface.

(g) The shell is of constant thickness h, i.e. $\lambda_3 = 1$.

(h) The shell material is nearly incompressible.

(i) Non-self-intersection and non-intersection with the vitteline membrane have to hold (enforced using a fast triangle-triangle intersection test[38]).

The material parameters are $\nu = 0.45$ (close to that expected for water-filled tissue), E = 100 Pascal, h = 0.5 μm, $\gamma = 20$ for VFI and 10 for the cephalic furrow, and ratio of bending stiffness of the mesoderm primordium to that of the passive tissue λ = 20 for VFI and 10 for cephalic furrow.

Specimen preparation and live imaging

Drosophila live imaging experiments were performed with embryos homozygous for the membrane label Spider-GFP and the nuclear label His2Av-RFP. This line was constructed by combining stocks of w*; P{w[+mC]=His2Av-mRFP}; + (Bloomington *Drosophila* Stock Center, 23560) and w; +; Spider-GFP[23]. Double labeled *Drosophila* embryos were dechorionated with 50% sodium hypochlorite solution (Sigma-Aldrich, 425044) and embedded in 1% low-melting temperature agarose (Lonza, SeaPlaque) in a 2 mm O.D. x 20 mm glass capillary (Hilgenberg GmbH) as has been previously described[25]. After polymerization, the agarose cylinder was extruded just enough to expose the embryo outside of the glass capillary. The capillary holding the embryo was mounted vertically within the water-filled recording chamber of the SiMView light sheet microscope. Images were acquired at 2-minute intervals. Each time point comprises two-color z-stacks recorded from four orthogonal optical views for two different physical orientations (dorso-ventral and lateral), encompassing the entire volume of the embryo with an axial step size of 1.950 μm. For all data presented, the recording was terminated when the larva hatched and crawled out of the field of view (**Supplementary Movie 2**), after which the larva was transferred to a standard vial of fly food and raised to adulthood.

Image processing and analysis

Prior to multiview fusion, raw SiMView recordings were corrected for insensitive pixels on the sCMOS detectors, using median and standard deviation filters. Multiview image fusion was

performed with a custom Matlab processing pipeline, using rigid transformation for multiview stack registration followed by linear blending[25].

Automatic segmentation of the membrane marker channel was performed with the watershed algorithm and subsequent agglomeration of oversegmented regions by persistence-based clustering[39]. To remove false positives, the nuclei marker channel was segmented with the same methodology, and a one-to-one matching between nuclei and membrane segmented regions was obtained using the Jaccard distance and the Hungarian algorithm[40]. Only objects that have a unique correspondence to the nuclei channel were assumed to correspond to cells and were used in the analysis. Cell segmentations were improved manually by an expert using itkSNAP. The centroids of the segmented cells were used to construct a point-cloud. Whole-embryo morphologies were obtained by interpolation of point-cloud data (**Supplementary Note 5**). Anisotropy with respect to the anterior-posterior axis was scored by approximating the cell outlines by ellipsoids, projecting the axes of these ellipsoids onto the plane parallel to the anterior-posterior axis, projecting the result onto the anterior-posterior axis and taking the ratio of largest to smallest projection.

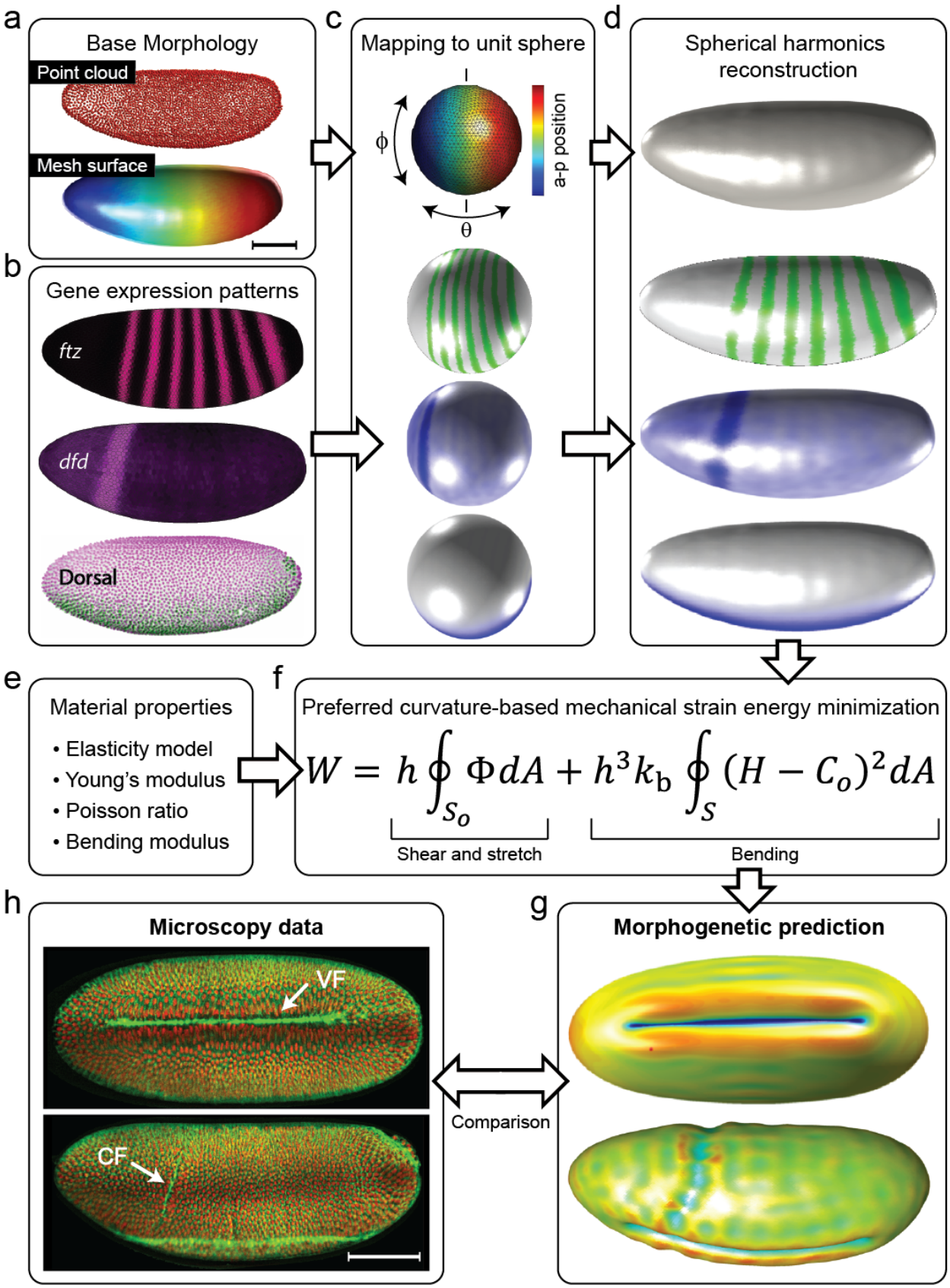

a
Base Morphology
Point cloud
Mesh surface
b
Gene expression patterns
ftz
dfd
Dorsal
c
Mapping to unit sphere
ϕ
θ
a-p position
d
Spherical harmonics reconstruction
e
Material properties
• Elasticity model
• Young's modulus
• Poisson ratio
• Bending modulus
f
Preferred curvature-based mechanical strain energy minimization
$W = h \oint_{S_o} \Phi dA + h^3 k_b \oint_S (H - C_o)^2 dA$
Shear and stretch
Bending
h
Microscopy data
VF
CF
Comparison
g
Morphogenetic prediction

Figure 1 | SPHARM-MECH overview

(**a**) Morphology data. Top: Cell centers from segmentation of the nuclear label His2Av-RFP channel from a three dimensional stack of a live SiMView light-sheet microscopy recording of a whole *D. melanogaster* embryo at the cellularized blastoderm stage. Bottom: surface triangular mesh based on cell centers. Color code anterior-posterior (y-axis) with colorbar as in (c)-top. (**b**) Gene expression data. Example gene expression patterns of *ftz*, *dfd* and *dl*. *ftz* and *dfd* patterns are displayed using PointCloudXplore[10]. (**c**) Spherical mapping. Cartesian coordinates (top: color code corresponds to (a) bottom with values provided in colorbar). Gene expression patterns of *ftz*, *dfd* and *dl* are mapped onto the unit sphere. (**d**) Spherical harmonics reconstruction (projection). Based on the spherical mapping in (c) Fourier coefficients are calculated for morphology and gene expression patterns to produce the internal analytical representation used by SPHARM-MECH (Online Methods). The outline is represented by 25 numbers (SPHARM coefficients). (**e**) Material properties data may be obtained from experimental measurements or estimated based on physical assumptions. (**f**) Energy minimization. Gene product activity increases the configuration-dependent strain energy by inducing local changes to preferred curvature. Morphology changes occur to relieve that energy (Online Methods and **Supplementary Note 6**). (**g**) Example SPHARM-MECH simulation results showing formation of the ventral furrow invagination (top) and simultaneous simulation of ventral and cephalic furrows (bottom). (**h**) Ventral and lateral views of maximum intensity projections of three dimensional stacks of SiMView microscopy recording of a whole *D. melanogaster* embryo, homozygous for the membrane label Spider-GFP and the nuclear label His2Av-RFP, 22 min after onset of gastrulation (see **Supplementary Videos 1** and **2** for complete time-lapse recording). Scale bars, 100 μm (a, h).

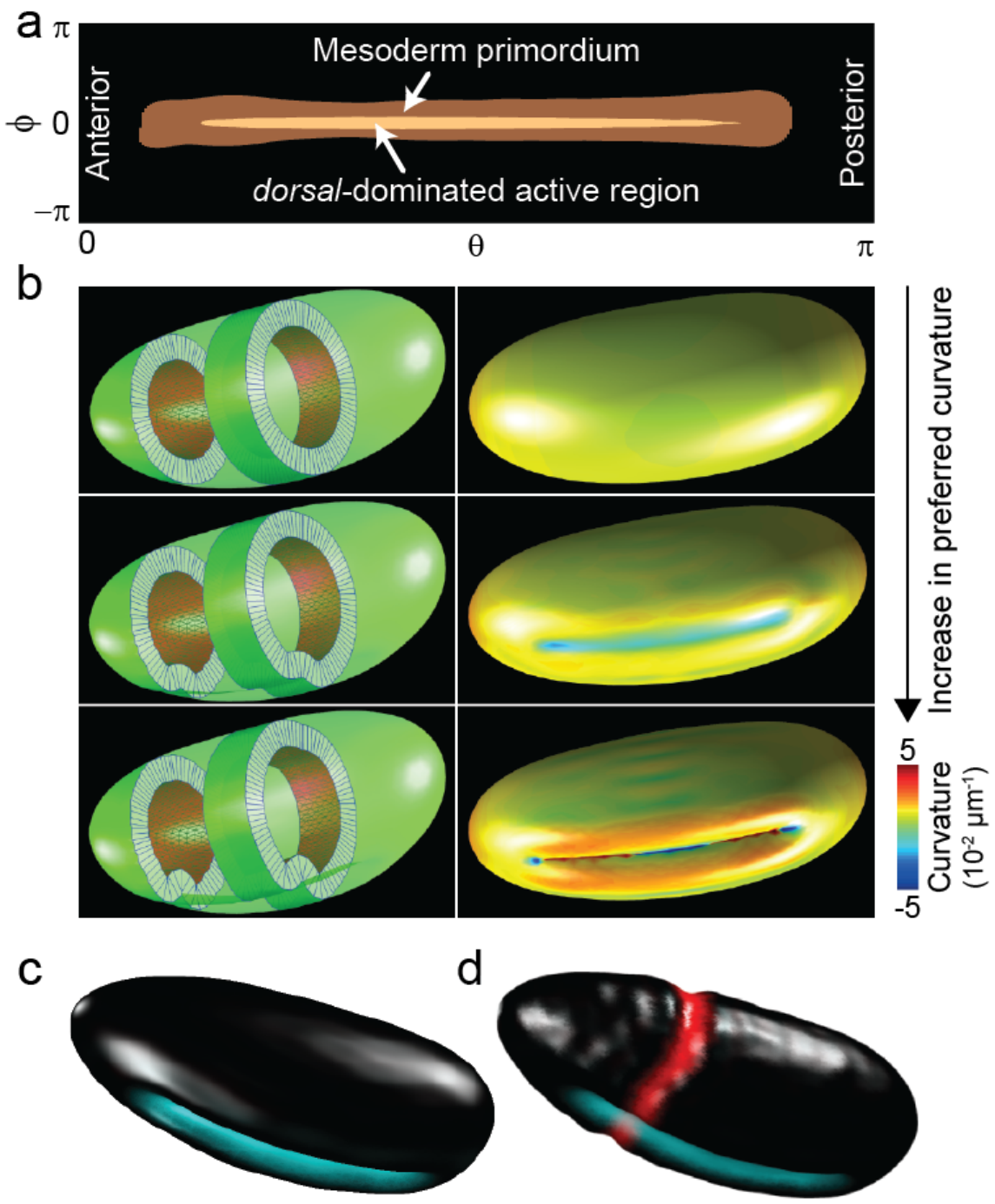

a
π
ϕ 0
−π
Anterior
Mesoderm primordium
dorsal-dominated active region
Posterior
0
θ
π
b
Increase in preferred curvature
5
-5
Curvature
(10-2 μm-1)
c
d

Figure 2 | Simulation of ventral and cephalic furrows

(**a**) Mesoderm primordium region: Threshold of map that results from the interaction of *snail*, *twist* and *huckebein*. Threshold was chosen so the mesoderm primordium covers 16% of the total area. Area in the center shows the 8-cell wide region defined as the activity map, based on *dorsal* and *huckebein* expression patterns, used for VFI simulations in (b and c) (**Supplementary Note 8**). Angles θ and ϕ represent the anterior-posterior and dorso-ventral positions respectively. (**b**) Left: Perspective views of VFI morphology predicted by SPHARM-MECH when gradually increasing the preferred curvature. Right: local mean curvature color code of corresponding morphologies on the left. (**c**) Perspective view of the bottom-most morphology of (b) with color showing the active region used. (**d**) Simultaneous simulation of VFI and CF. Colors mark the active regions.

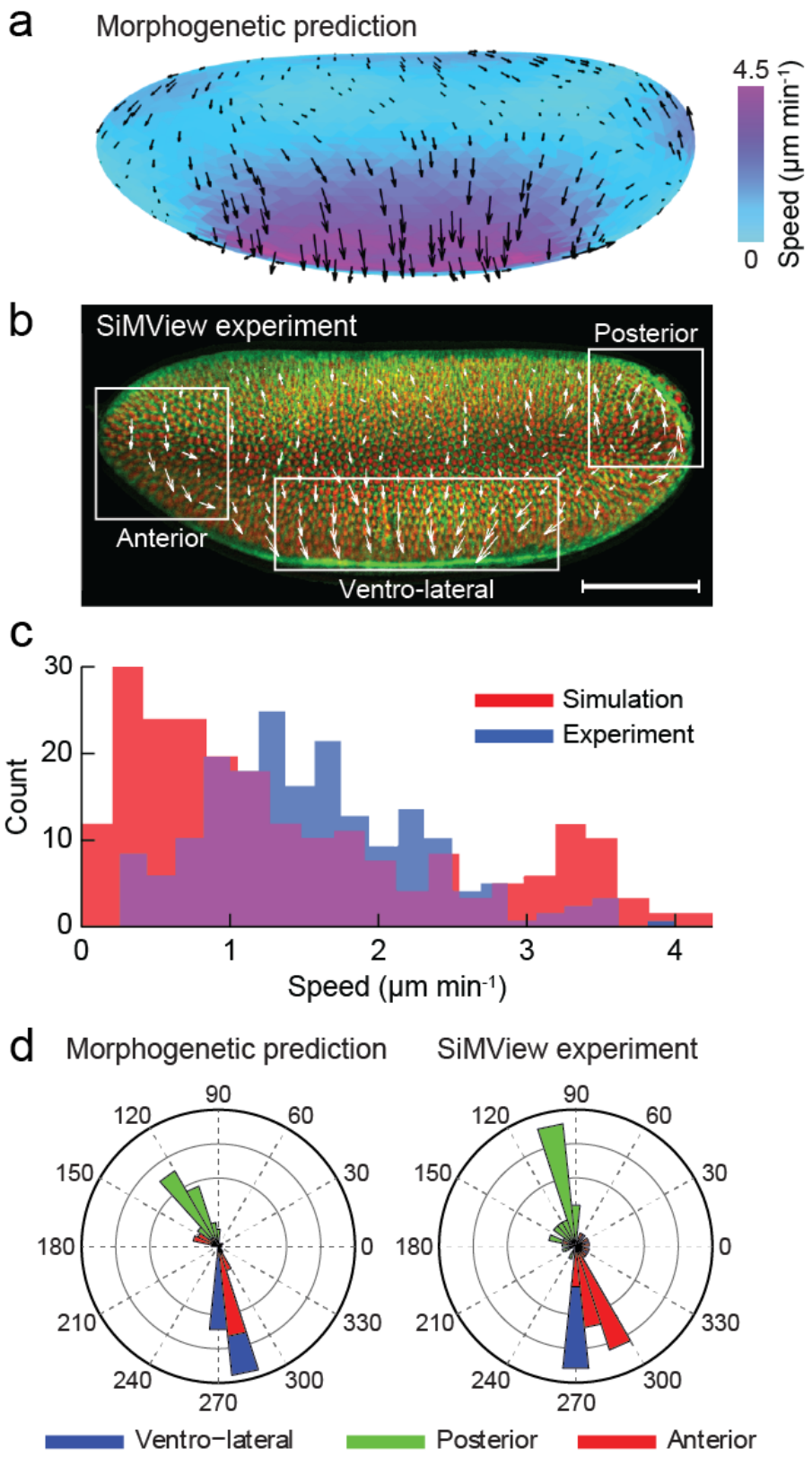

a
Morphogenetic prediction
4.5
0
Speed (μm min-1)
b
SiMView experiment
Posterior
Anterior
Ventro-lateral
c
Simulation
Experiment
Count
30
20
10
0
0
1
2
3
4
Speed (μm min-1)
d
Morphogenetic prediction
SiMView experiment
90
60
30
0
330
300
270
240
210
180
150
120
Ventro−lateral
Posterior
Anterior

Figure 3 | Comparison of experimentally observed and simulated tissue flows

(**a**) Tissue flow velocity field obtained from a VFI-only simulation showing speeds (color code and relative arrow size) and direction of flow. Results represent a simulation from undeformed geometry to one that equals the furrow after 10 minutes of onset of gastrulation. (**b**) Experimentally observed tissue flow velocity field obtained by manual tracking of 270 cell centroids and interpolated over the image over 10 minutes of onset of gastrulation. (**c**) Speed histogram corresponding to simulation and experiment. (**d**) Flow direction histogram angular plots corresponding to simulation (left) and experiment (right), showing resulting flow directions in the three regions outlined in (b). Scale bar, 100 μm.

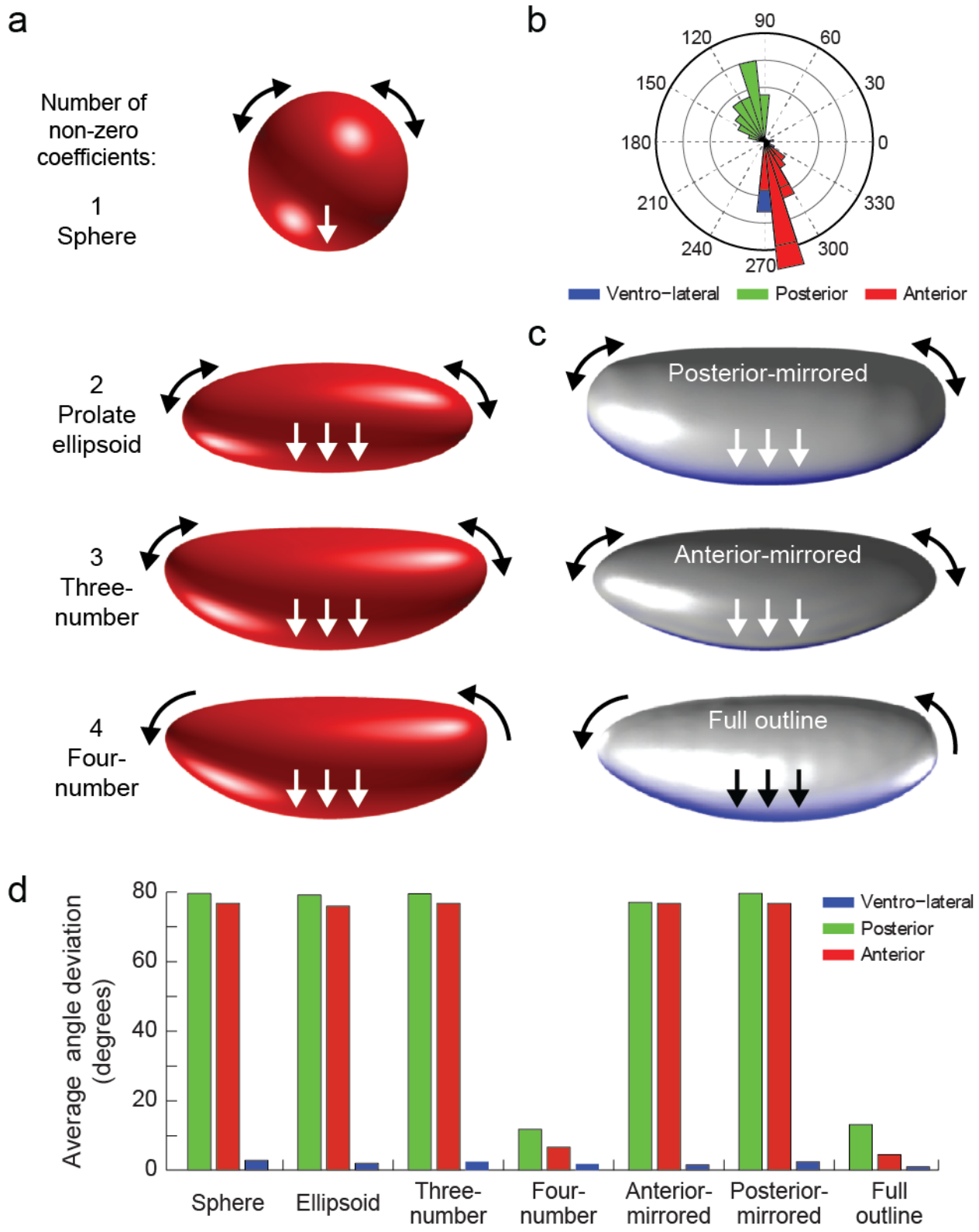

a
Number of non-zero coefficients:
1 Sphere
2 Prolate ellipsoid
3 Three-number
4 Four-number
b
90
60
30
0
330
300
270
240
210
180
150
120
Ventro−lateral
Posterior
Anterior
c
Posterior-mirrored
Anterior-mirrored
Full outline
d
Average angle deviation (degrees)
80
60
40
20
0
Ventro−lateral
Posterior
Anterior
Sphere
Ellipsoid
Three-number
Four-number
Anterior-mirrored
Posterior-mirrored
Full outline

Figure 4 | Long-range effects of embryo symmetry on tissue flows resulting from ventral furrow invagination

(**a**) Four SPHARM-MECH coefficients are sufficient to approximate the *D. melanogaster* blastoderm outline. Each coefficient breaks a different type of symmetry as seen when going from top (sphere) to bottom (four-coefficient shape). Please see **Supplementary Note 7** for more details regarding the meaning of the coefficients. When using the top three shapes (that possess left-right [anterior-posterior] symmetry) as base shapes for performing the VFI simulation instead of the full outline, tissue regions on the posterior (defined arbitrarily to be located on the right) flow dorsally only 50% of the time and ventrally otherwise. This is indicated by the black two-headed arrows, showing that the flow can go in either direction. In addition, the anterior and posterior flows are always with opposite destinations, i.e. when posterior tissue flows dorsally, anterior pole tissue flows ventrally and vice versa. However, when the anterior-posterior symmetry is broken (the four-number shape), the posterior (lower curvature) pole region tissue flows dorsally in all of the simulations (n = 6). This is indicated by the black single-headed arrows. Flow ventrally (towards the forming invagination) from the lateral sides is present in all cases. This is shown by the white arrows. (**b**) Flow direction histogram angular plot corresponding to a typical simulation with the sphere as base undeformed shape. Assuming the pole where tissue flows dorsally to be posterior, this shows flow directions similar to the ones observed in the experiment (and simulation with the full outline) (**Fig. 3d**). Similar results are obtained for all simulations. (**c**) Two cases were constructed: a left-right symmetric outline (top) with the posterior features preserved (posterior-mirrored) and another (middle) with the anterior features preserved (anterior-mirrored). Similar to the left-right symmetry cases in (a), the flow was observed in each direction 50% of the time. However, when performing the VFI simulation on the full morphology repeatedly, the flow was always from the posterior dorsally, indicating that it is indeed the break in left-right symmetry that biases the direction of flow. (**d**) Plot of average (n = 6) flow angle deviation with respect to the experimentally observed flow. Only the simulations with shapes that possess the left-right asymmetry yield differential tissue flows close to the experimental observation.

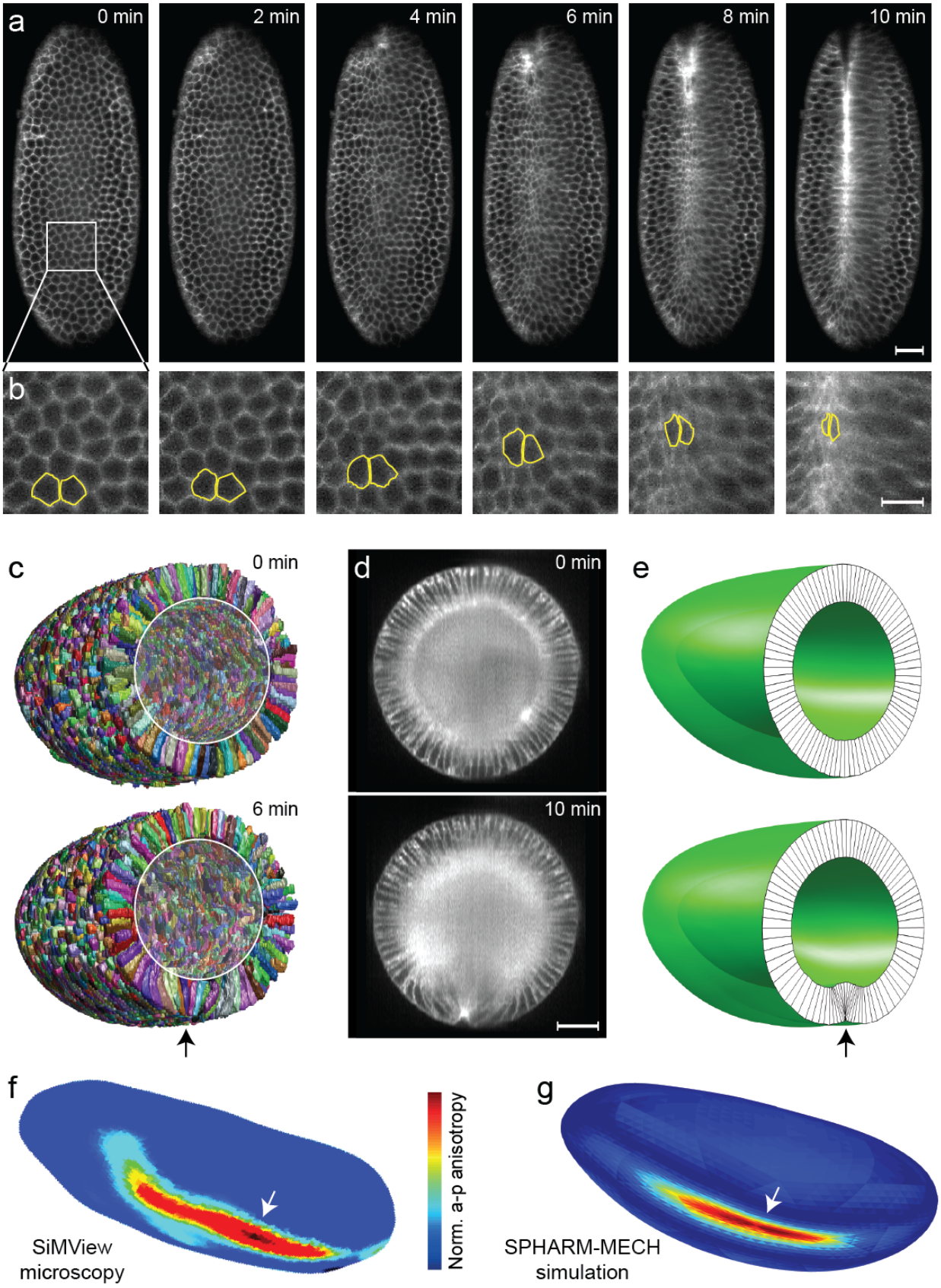
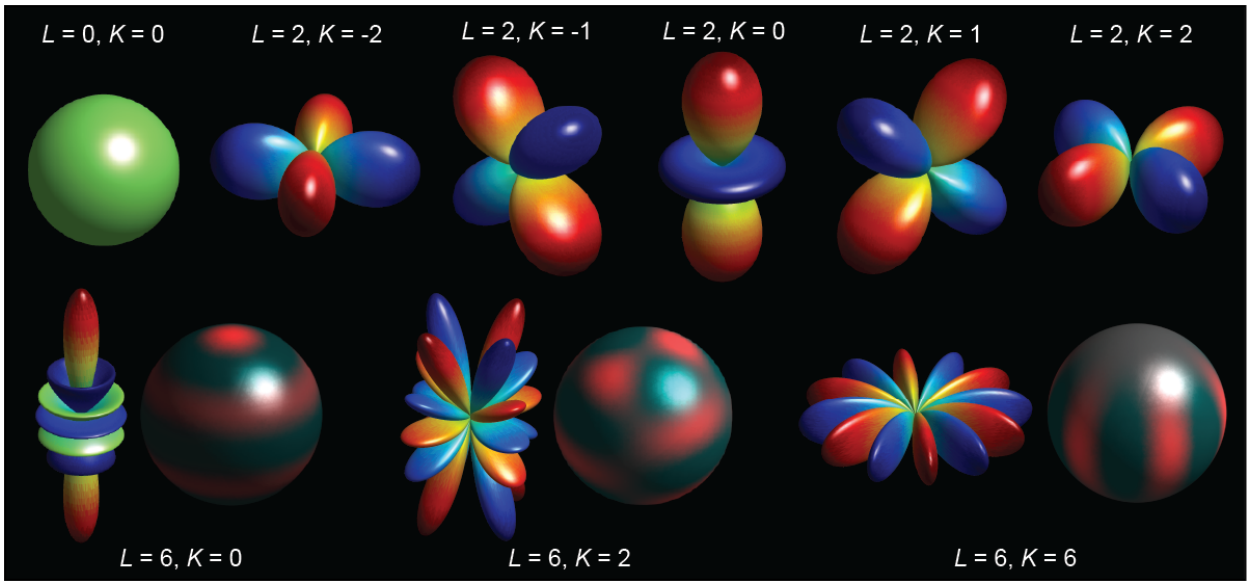

a
0 min
2 min
4 min
6 min
8 min
10 min
b
c
0 min
6 min
d
0 min
10 min
e
f
SiMView
microscopy
Norm. a-p anisotropy
g
SPHARM-MECH
simulation

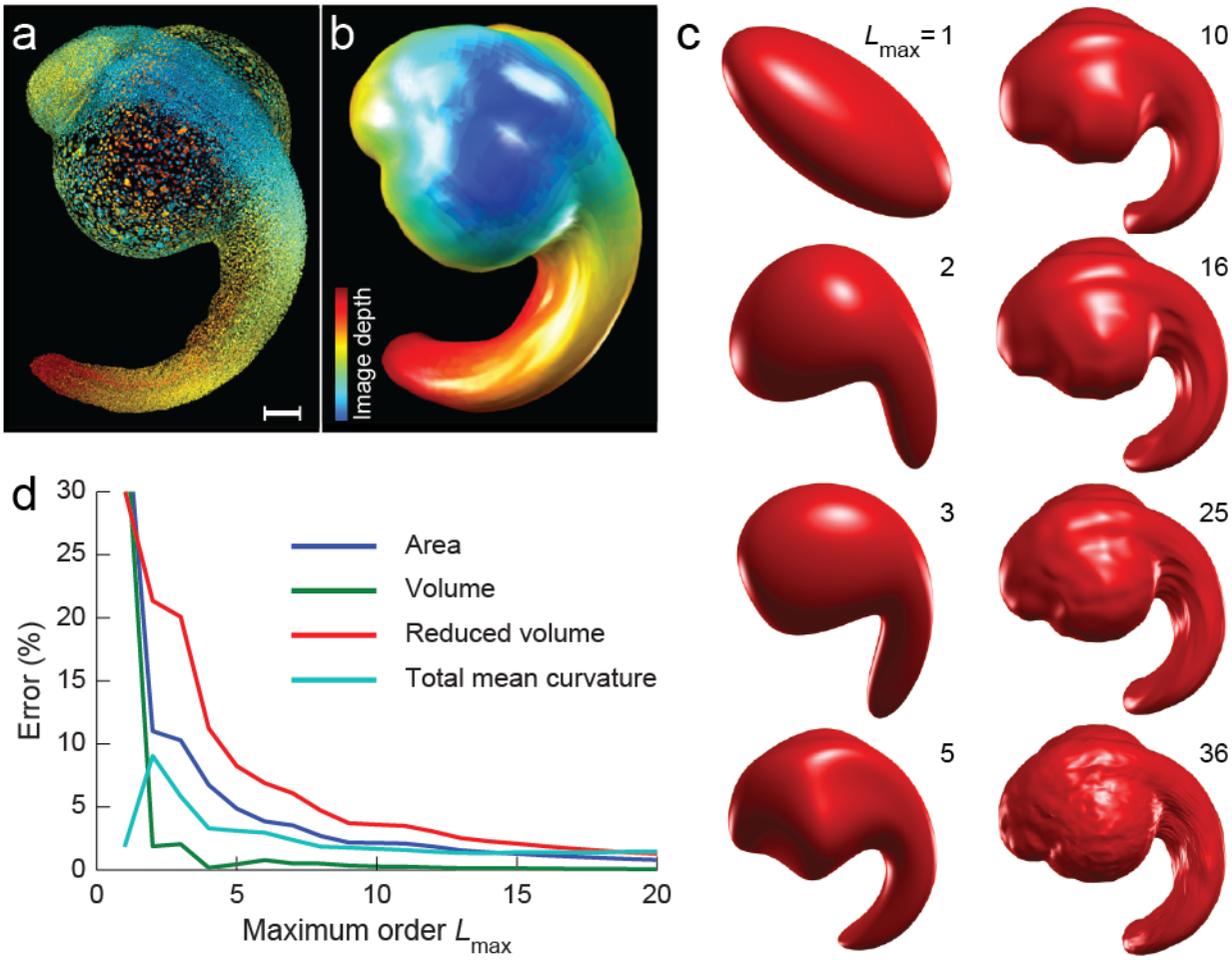
Figure 5 | Comparison of experimentally observed and simulated tissue anisotropy

(**a**) Time sequence of Spider-GFP SiMView light-sheet microscopy images. Whole-embryo three-dimensional stacks were recorded (**Supplementary Video 1**). Only one plane, which approximately cuts through the ventral furrow of each stack, is shown, with the anterior pole at the top. (**b**) Enlarged image of the region marked in (a) of the same time sequence, following the outlines of two neighboring cells (yellow outlines), and showing the development of anisotropy as the ventral furrow invagination forms. (**c**) Segmentation results for time points 0 min and 6 min. Black arrow: beginning of furrow formation. (**d**) SiMView light-sheet microscopy images of Spider-GFP (membrane label) embryos. Approximately the middle planes of the three-dimensional stacks at time 0 min and 10 min are shown. (**e**) Corresponding simulation cut-through surfaces when the preferred local mean curvature of the mid-plane surface at the active region is increased from -0.5 (top) to 1.5 (bottom). Lines are shown as visual guides to the simulated deformation and should not be understood as cell boundaries. Black arrow: beginning of furrow formation. (**f**) Whole blastoderm morphology in perspective view with color code equal to a-p anisotropy (normalized). White arrow: beginning of furrow formation, corresponding to region indicated by black arrow in (c). (**g**) Same as (f) performed for the simulated morphology. White arrow: beginning of furrow formation, corresponding to region indicated by black arrow in (e). Scale bars, 20 µm (a), 10 µm (b), 50 µm (d).


L = 0, K = 0
L = 2, K = -2
L = 2, K = -1
L = 2, K = 0
L = 2, K = 1
L = 2, K = 2
L = 6, K = 0
L = 6, K = 2
L = 6, K = 6

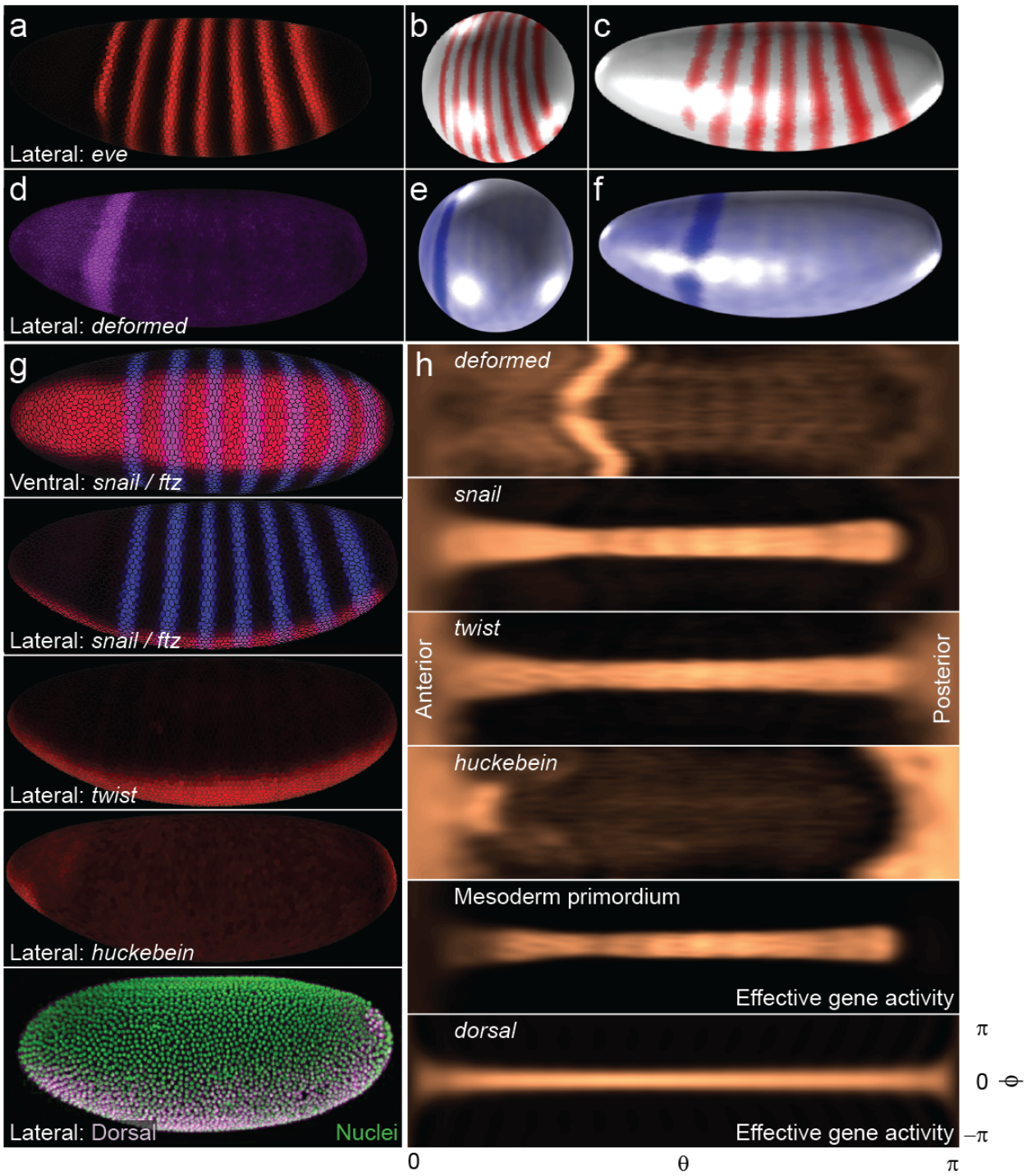
Supplementary Figure 1 | Spherical harmonics basis functions

Examples of spherical harmonics basis functions (**Supplementary Note 1**). These functions are well known as the angular portion of a set of solutions to Laplace's equation. Color code: blue negative to red positive values. In the bottom row a perspective view of the corresponding mapping of function values to the unit sphere is shown.


a
b
Image depth
c
L_{max} = 1
10
2
16
3
25
5
36
d
Area
Volume
Reduced volume
Total mean curvature
Error (%)
Maximum order L_{max}
0
5
10
15
20
25
30

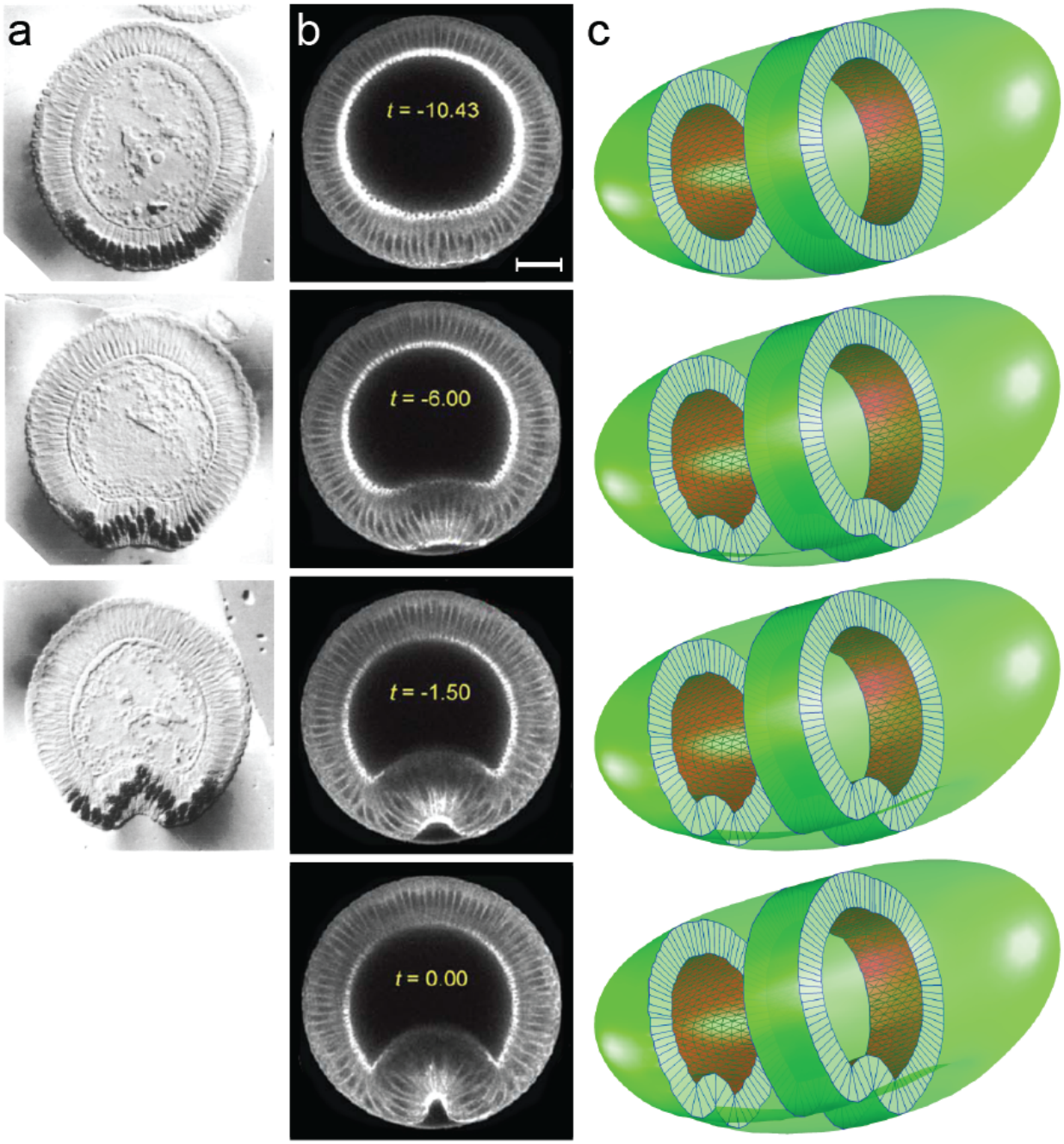
Supplementary Figure 2 | SPHARM representation of a zebrafish embryo

(**a**) Maximum-intensity projection of a SiMView image stack showing a 22 hours post fertilization zebrafish embryo. Color indicates depth into the image. (**b**) SPHARM-MECH reconstructed surface of (a) with maximum order L_{max} = 20. (**c**) Reconstructed surfaces using increasing L_{max}. (**d**) Convergence of the spherical harmonics series (fidelity to the data): plot of percent error of surface properties of original surface mesh reconstruction *vs.* maximum order L_{max} used in fitting spherical harmonics coefficients. Scale bar, 100 µm.


a
Lateral: eve
b
c
d
Lateral: deformed
e
f
g
Ventral: snail / ftz
Lateral: snail / ftz
Lateral: twist
Lateral: huckebein
Lateral: Dorsal
Nuclei
h
deformed
snail
twist
Anterior
Posterior
huckebein
Mesoderm primordium
Effective gene activity
dorsal
Effective gene activity
π
0
−π
ϕ
0
θ
π

Supplementary Figure 3 | Mapping of gene expression patterns and determination of mesoderm primordium and active regions

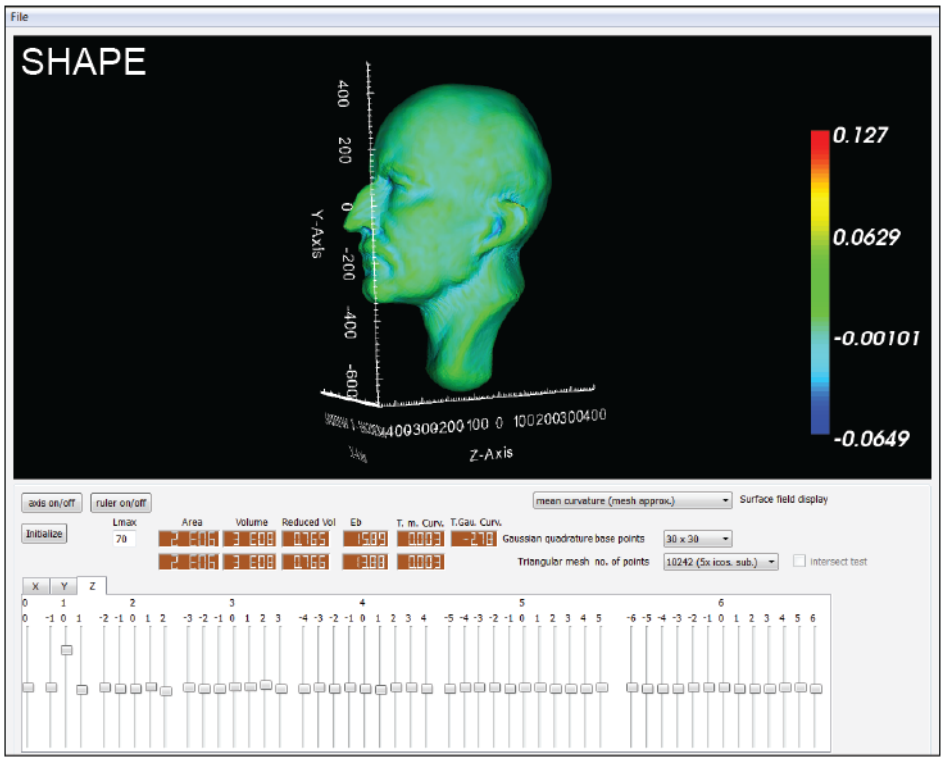
(**a**) Expression pattern of *eve*. (**b**) Mapping of *eve* to the unit sphere. (**c**) SPHARM-MECH representation of *eve* shown on the full blastoderm morphology. (**d-f**) same as a-c but for *deformed* (*dfd*) which is used to define the region of activity for the cephalic furrow formation. (**g**) From top to bottom: ventral and lateral views of *ftz* and *snail* patterns, lateral view *twist*, lateral view *huckebein* and lateral view *dorsal*. Data for *eve*, *dfd, snail*, *twist* and *huckebein* is obtained from the Berkeley Virtual Embryo Project and displayed using PointCloudXplore Light. (**h**) Two-dimensional angular plots of the spherical harmonics representation of *dfd*, *snail*, *twist* and *huckebein* (top four panels), the interaction map of *snail*, *twist* and *huckebein* (**Supplementary Note 8**), and (bottom most) the pattern of *dorsal*.


a
b
c
t = -10.43
t = -6.00
t = -1.50
t = 0.00

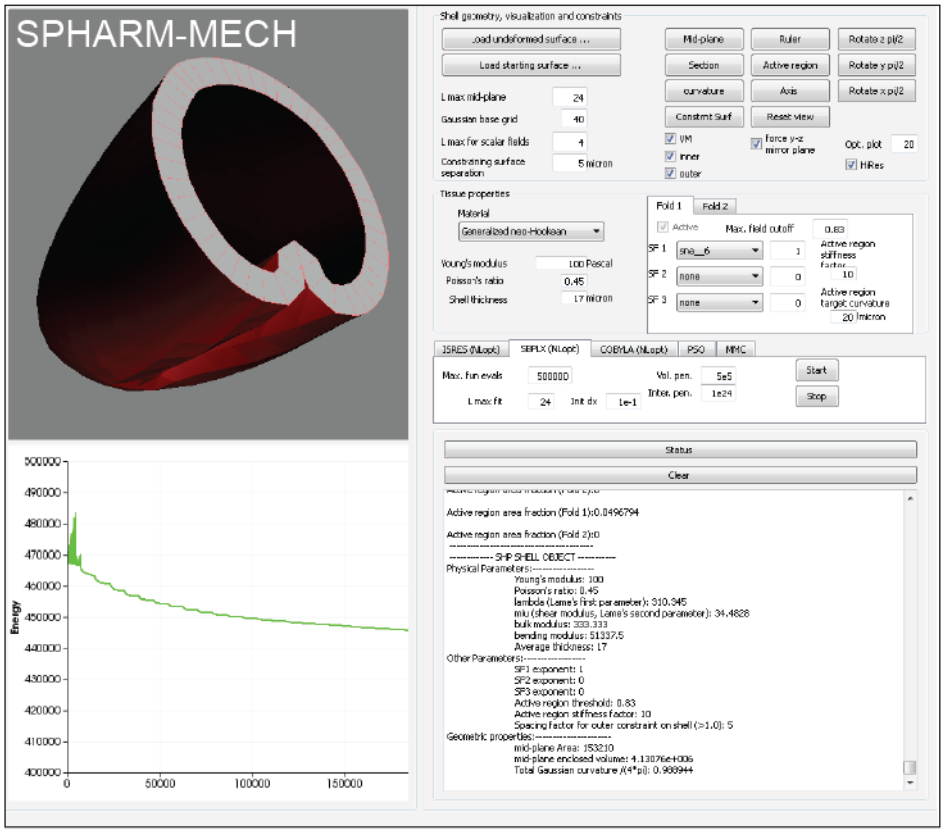
Supplementary Figure 4 | Ventral furrow invagination images compared to SPHARM-MECH simulation results

(**a**) *D. melanogaster* embryo electron microscopy images (adapted from Fig. 2 in Leptin and Grunewaldt[27]), and (**b**) multi-photon microscopy images of transgenic Sqh-GFP embryos (adapted from Fig. 6 in Conte *et al.*[14]). **(c)** SPHARM-MECH simulation of VFI showing a sequence of gradually increasing preferred curvatures when using the *dorsal*-based activity map of **Fig. 2a**. Scale bar, 20 µm.

a


File
SHAPE
0.127
0.0629
-0.00101
-0.0649
Y-Axis
Z-Axis
axis on/off
ruler on/off
Initialize
Lmax
Area
Volume
Reduced Vol
Eb
T. m. Curv.
T.Gau. Curv.
mean curvature (mesh approx.)
Surface field display
Gaussian quadrature base points
30 x 30
Triangular mesh no. of points
10242 (5x icos. sub.)
intersect test

b


SPHARM-MECH
Shell geometry, visualization and constraints
Load undeformed surface ...
Load starting surface ...
Mid-plane
Ruler
Section
Active region
curvature
Axis
Constrnt Surf
Reset view
Rotate z pi/2
Rotate y pi/2
Rotate x pi/2
L max mid-plane
Gaussian base grid
L max for scalar fields
Constraining surface separation
Tissue properties
Material
Generalized neo-Hookean
Young's modulus
Poisson's ratio
Shell thickness
Fold 1
Fold 2
Active
Max. field cutoff
Active region stiffness factor
Active region target curvature
Max. fun evals
Vol. pen.
Inter. pen.
Start
Stop
Status
Clear
Active region area fraction (Fold 1):0.0496794
Active region area fraction (Fold 2):0
Physical Parameters:
Other Parameters:
Geometric properties:
Energy

Supplementary Figure 5 | Screenshots of SHAPE and SPHARM-MECH

(**a**) **SHAPE** (**S**pherical **HA**rmonic **P**arameterization **E**xplorer) is a Windows 64bit compiled program with a graphical user interface (GUI) that allows the user to view and modify SPHARM shapes that have been saved in the ".shp3" format. ".shp3" stores morphology and scalar fields such as gene expression patterns in the form of spherical harmonics coefficients. The program also demonstrates calculation of surface properties with Gaussian quadrature in comparison to usage of the triangular mesh. The SHAPE GUI uses the QT and VTK (Visualization Toolkit) C++ libraries. (**b**) **SPHARM-MECH** (SPHARM-Mechanics) is a Windows 64bit compiled program with a GUI that allows the user to import shapes in the ".shp3" format, to configure a tissue shell mechanics calculation and to execute one of several numerical optimization algorithms. The result is a shape prediction. The screenshot shows a predicted VFI morphology at the end of an optimization using the SubPlex algorithm.