natureresearch

Peer Review Information

Journal: Nature Methods
Manuscript Title: TubULAR: Tracking in toto deformations of dynamic tissues via constrained maps
Corresponding author name(s): Noah Mitchell

Editorial Notes: n/a

Reviewer Comments & Decisions:

Decision Letter, initial version:

Dear Dr Mitchell,

Your Article, "TubULAR: Tracking in toto deformations of dynamic tissues via constrained maps", has now been seen by 3 reviewers. As you will see from their comments below, although the reviewers find your work of considerable potential interest, they have raised a number of concerns. We are interested in the possibility of publishing your paper in Nature Methods, but would like to consider your response to these concerns before we reach a final decision on publication.

We therefore invite you to revise your manuscript to address these concerns. In particular, please ensure that the manuscript can be understood by a broad audience, especially biologists. To this end, you could move particularly lengthy technical details to a Supplementary Note. We also strongly recommend including a supplementary protocol explaining how to use TubULAR.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

When revising your paper:

* include a point-by-point response to the reviewers and to any editorial suggestions

* please underline/highlight any additions to the text or areas with other significant changes to facilitate review of the revised manuscript

* address the points listed described below to conform to our open science requirements

* ensure it complies with our general format requirements as set out in our guide to authors at www.nature.com/naturemethods

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We hope to receive your revised paper within eight weeks. If you cannot send it within this time, please let us know. In this event, we will still be happy to reconsider your paper at a later date so long as nothing similar has been accepted for publication at Nature Methods or published elsewhere.

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To further increase transparency, we encourage you to provide, in tabular form, the data underlying the graphical representations used in your figures. This is in addition to our data-deposition policy for specific types of experiments and large datasets. For readers, the source data will be made accessible directly from the figure legend. Spreadsheets can be submitted in .xls, .xlsx or .csv formats. Only one (1) file per figure is permitted: thus if there is a multi-paneled figure the source data for each panel should be clearly labeled in the csv/Excel file; alternately the data for a figure can be included in multiple, clearly labeled sheets in an Excel file. File sizes of up to 30 MB are permitted. When submitting source data files with your manuscript please select the Source Data file type and use the Title field in the File Description tab to indicate which figure the source data pertains to.

Please include a “Data availability” subsection in the Online Methods. This section should inform readers about the availability of the data used to support the conclusions of your study, including accession codes to public repositories, references to source data that may be published alongside the paper, unique identifiers such as URLs to data repository entries, or data set DOIs, and any other statement about data availability. At a minimum, you should include the following statement: “The data that support the findings of this study are available from the corresponding author upon request”, describing which data is available upon request and mentioning any restrictions on availability. If DOIs are

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We request that you deposit code in a DOI-minting repository such as Zenodo, Gigantum or Code Ocean and cite the DOI in the Reference list. We also request that you use code versioning and provide a license.

For more information on our code sharing policy and requirements, please see: https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-computer-code

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Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further. We look forward to seeing the revised manuscript and thank you for the opportunity to consider your work.

Sincerely,
Madhura

Madhura Mukhopadhyay, PhD
Senior Editor
Nature Methods

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
Tissue cartography makes shape change in biological image data tractable in cases where the relevant dynamics occur on a tissue surface by mapping the tissue surface to two dimensional images.
A limitation so far has been effective global (aka in toto) mapping of complex dynamic surfaces, with applications so far either focusing on global mapping of relatively simple static shapes like the early Drosophila embryo, or on local patches of complex dynamic shapes.
Furthermore, computation of tensor operations on tissue surfaces had not been fully worked out, for example, gradients of rotation of vectors.
Here the authors solve this problem for unbranched, simply connected surfaces of arbitrary geometry.
In particular they develop a strategy to create global maps in material coordinates that minimze tissue movement in the map and implement tensor operations using discrete differential geometry.

This is a major improvement of current tissue cartography methods that should be of broad interest to readers of Nature Methods.
The analysis of the shape change of the beating heart is elegant and very nicely done.
However, to ensure broad impact, the manuscript would benefit from more detailed illustration of applications to morphogenesis, and the installation procedure of the tool needs to be simplified.
In particular, although the authors made a very helpful website describing how to use their tool, I was not able to get either of their two main examples to run. I provide a description of my problems below

Some specific comments:
- The main manuscript occassionally gets lost in distracting technical details. For example a description of level sets seems unnecessary. Moreover, from looking at Fig S1 it seems that the level sets generally rely on first running Ilastik segmentation and so are really just smoothing the surface of a 3D segmentation in Ilastik. That is completely fine but not stated clearly anywhere, leaving the reader to puzzle what kind of image data is needed for the level set methods to work properly, because using the image as a potential/cost function directly is unlikely to work for arbitray image data.
- In any case, surface detection was working well in previous incarnations of tissue cartography and the innovation of this manuscript lies in the definitions of the material coordinates and implementation of tensor operations. Yet the description of how material coordinates are defined conceptually could be much clearer and perhaps even deserves a main figure.
- Similarly, details about different conformal mapping algorithms can be moved to methods supplements.
- It is a little disappointing that what is seemingly the main application of the tool, to make epithelial morphogenesis tractable, is left as a bit of a footnote with the main manuscript only stating: “Separating out the effects of tissue motion and cell intercalation has given insights into multiple mechanisms” and delegating an illustration to a short section at the end of the supplement which is hard to follow.
- The fact that there is comparison with previous methods to illustrate the improvement by tubular in the supplement is great, but it is actually not very clear that there are artefacts where the red arrows in Fig S9 are, perhaps this requires insets and/or further explanation.
- In fig 4B it would be nice to see the area contraction visualized in the same way as the normal velocity, perhaps it could replace the phase of the tangential velocity in the same panel.
- The math supplement is probably only readable by those that already know the math involved, and that is entirely reasonable, but it would be helpful to slightly dejargonize / help the rusty reader remember certain definitions. For example homotopically could add something like (smoothly, topology preserving), etc.

In summary, this is great work that could find broad application if it were a little easier to read and use.

Description of problems in getting the examples to run:
——

Running example_timeseries_gut11Timepoints.m, in the block %% EXTRACT CENTERLINES, I got the error:

Undefined function 'perform_front_propagation_3d' for input arguments of type 'double'.

Error in perform_fast_marching (line 121)
[D,S,Q] = perform_front_propagation_3d(W,start_points-1,end_points-1,nb_iter_max, H, L, values);

Error in TubULAR/generateFastMarchingCenterlines (line 379)
[D2,S] = perform_fast_marching(DD, startpt_transposed, options);

Error in example_timeseries_gut11Timepoints (line 409)
tubi.generateFastMarchingCenterlines(cntrlineOpts)

I then went to "Common Debugging Issues" on the website and found the solution: "This is a function that is inside gptoolbox, in the external folder. Make sure you run GPToolbox's external/toolbox_fast_marching/compile_mex.m successfully, run with MATLAB from within the parent directory (ie the current working directory should be something like tubular/external/gptoolbox/external/toolbox_fast_marching/)."

So I followed the instruction to install that, but unfortunately failed because of a cmake error:

cmake ..
-- The C compiler identification is AppleClang 12.0.5.12050022
-- The CXX compiler identification is AppleClang 12.0.5.12050022
-- Detecting C compiler ABI info
-- Detecting C compiler ABI info - done
-- Check for working C compiler: /Library/Developer/CommandLineTools/usr/bin/cc - skipped
-- Detecting C compile features
-- Detecting C compile features - done
-- Detecting CXX compiler ABI info
-- Detecting CXX compiler ABI info - done
-- Check for working CXX compiler: /Library/Developer/CommandLineTools/usr/bin/c++ - skipped
-- Detecting CXX compile features

-- Detecting CXX compile features - done
CMake Error at /Applications/CMake.app/Contents/share/cmake-3.24/Modules/FindPackageHandleStandardArgs.cmake:230 (message):
Could NOT find Matlab (missing: Matlab_INCLUDE_DIRS Matlab_MEX_LIBRARY Matlab_MEX_EXTENSION Matlab_ROOT_DIR MEX_COMPILER MX_LIBRARY ENG_LIBRARY)
(found version "NOTFOUND")
Call Stack (most recent call first):
/Applications/CMake.app/Contents/share/cmake-3.24/Modules/FindPackageHandleStandardArgs.cmake:594 (_FPHSA_FAILURE_MESSAGE)
cmake/FindMatlab.cmake:1513 (find_package_handle_standard_args)
CMakeLists.txt:62 (find_package)

-- Configuring incomplete, errors occurred!
See also "/Users/XX/repos/tubular/external/gptoolbox/mex/build/CMakeFiles/CMakeOutput.log".
See also "/Users/XX/repos/tubular/external/gptoolbox/mex/build/CMakeFiles/CMakeError.log".

The website says: "If this runs into trouble, it is possible that you may have to tweak the CMake file depending on your computer specs. StackExchange is a place to look for any errors you might get."
Unfortunately, this was too much for me. I tried and found the suggestion to run "export Matlab_ROOT_DIR=/usr/local/MATLAB/R2020b/" but that did not fix it.
In case it is helpful, this is on MacOs 11.6 running Matlab 2020b.

I then attempted to run example_singlecoil.m

First I got the error:

Error using cd
Cannot CD to /Users/XX/repos/tubular/example/example_singlecoil.m (Name is nonexistent or not a directory).

and several others that were all fixed by copying equivalent block from example_timeseries_gut11Timepoints.m
but then I ran into an error without an obvious quick fix:

defining TubULAR class instance (tubi= tubular instance)
Reference to non-existent field 'im_pivPathlines'.

```
Error in TubULAR/initializeTubULAR (line 582)
tubi.fullFileBase.im_pivPathlines = fullfile(tubi.dir.im_pivPathlines, tubi.fileBase.im_pivPathlines)
;

Error in TubULAR (line 233)
tubi.initializeTubULAR(xp, opts)

Error in example_singlecoil (line 258)
tubi = TubULAR(xp, opts) ;
```

Reviewer #2:
Remarks to the Author:
See uploaded attachment.

[attached]

In this work, Mitchell and Cislo present a comprehensive framework for analysing complex biological tissue shapes. They provide a powerful suite of tools that enable the user to quantify the morphological properties of tubular-like structures (of which there are many in biology). The work builds on previous tissue cartography approaches, but generalises the approach to be more robust and cope better with tissue deformations. The paper is well written and clear to follow. Further, the Supplementary Material is presented in a logical manner and approachable.

The online documentation is very well curated and accessible. I tested the software for the midgut example. This worked well (though slowly on my computer). Though I don't think anyone can just use this software quickly (it's not simply a click and play software), someone with a sensible level of computing experience should be able to get the software working reasonably quickly.

Originally, I was somewhat sceptical that this work represented a substantial advance on Heemskerk *et al.* (Nat Meth 2015). However, the combination of surface visualisation, conformal mapping and exterior calculus modules all within one framework is very powerful. Therefore, I think this work is suitable for a broad journal such as Nature Methods. However, having said that, I am not convinced it merits a full 5-figure article (discussed further below).

Major comments

1. Heemskerk and Streichan was a Brief Communication. I feel that likewise this is a more apt format for this paper. I hold this view for two reasons. First, the figures could be substantially streamlined. For example, Figures 1 and 2 are highly related and don't really merit separate representation – as they're both laying out the fundamentals behind TubULAR. Second, while the approaches outlined are very powerful in combination, each separately does not represent a major conceptual advance. The ideas here have already been developed in different contexts. The power of this work is bringing those into a coherent framework and applying to biology relevant problems; doing so requires less space than if describing new theoretical approaches. I am not fixed in this view, but better justification of the intellectual and technical advances are needed if the authors think it should stay as a longer format.

2. Looking at Figure S8, the error in the scalar field gradient appears surprisingly large, especially compared to the other measures. Can the authors provide an explanation for this and does this impact the results (I would imagine that error over 10% could well affect the measures).

3. As a tool, it needs to be accessible to biologists. An important point here is how "tube-like" a surface needs to be. It would be helpful to provide an example of a tissue that *does not work.* This will help readers to better appreciate which systems TubuLAR is good for and which it isn't.

4. How end caps can be defined remains somewhat unclear. What are the limitations on defining such caps? Relatedly, is there a minimal size for the approach to work – *e.g.* could it even work on describing a single cell? I assume that at some point the discretised approximation of spatial derivatives stops being a good approximation.

5. The implementation of DECLab and the decompositions are impressive. While it seems the code works well, it is another thing to interpret the results. Given this is for a broad readership, it will be helpful to give some specific biological examples of certain measures. For example, for the Helmholtz-Hodge decomposition, what does a "dilatational" and "harmonic" parts actually reflect in terms of the heart morphology?

Minor comments

Page 3: Typo: "… a given time point, we the point cloud …" Meaning unclear

Drosophila should be *Drosophila* [italicised]

Is the heart really imaged in superresolution [*i.e.* resolution < Abbe limit]. I am not sure that's the case for the given data.

Reviewer #3:
Remarks to the Author:
This manuscript by Drs. Mitchell and Cislo describes a methodology to capture, quantify and describe in toto measurements of tissue deformation of tubular architectures across time. They execute this through TubULAR, an open-source MATLAB framework they have developed and have freely shared. This work advances the current state of the art and integrates with the ImSAnE framework. Overall, this is a well-written manuscript, which when read in conjunction with the Supplementary Material provides detail of the approaches used and the rationale for the process chosen. The utility of this framework is high and significant, with illustrations of the applicability in two systems with dramatically different deformation and time-dependent behaviors in the developing Drosophila midgut and zebrafish heart. The fundamental challenge with this manuscript is the lack of comparative quantitative measures to determine if the solution that is generated from the method is correct/accurate. The images and pullback maps seem reasonable, and the methods and calculations performed should be correct. However, it is unclear if TubULAR provides an accurate representation of the real system or where/what cases this approach fails or larger errors are generated. Additionally, other than cartoon generalized examples comparing the approach used in the TubULAR framework relative to other methods, could there be a better quantitative description of the performance of TubULAR relative to IMSAnE for the overlapping functionality? This latter point is important as the authors specifically point out some selected failures with IMSAnE's approach as a generalized critique, but it would be beneficial for readers to understand the outcomes of the difference in error/accuracy from the different methodologies. With these details integrated, I believe this would be an important technological development that would be beneficial to the community.

Additional specific suggestions to the authors to improve the manuscript:

- Description of the methods used to capture the midgut data should be provided. This was done later for the heart dataset. What is the marker that is being used to identify the midgut? The authors note that there is a monolayer of endoderm surrounded by a thin net of muscle cells. How was segmentation performed? The use of the level set method implies a discreet cell/tissue label, but it is unclear how this was done.
- The imaging modalities this framework is compatible with should be explicitly described. The beginning of the manuscript focuses on descriptions of segmenting and constructing tissue surfaces from 3D data. While inferred from the beginning of the manuscript based on the mention of voxels, and later in the description of lightsheet imaging for the zebrafish heart dataset, explicit discussion of compatible image data architectures would be valuable to the reader. i.e. this method cannot be directly applied to confocal z-stacks, 2D data to obtain 3D information, pixel structure vs voxels.

- At the end of the manuscript, there are claims of documented examples of this process having been used for neural tube and tracking a phase-separated droplet in a microtubule gel. Minimal information and no detail on method was described in the supplementary info for the latter (figure ref label is also incorrect), with methods referring to a paper that has been “submitted” by a group that is not the authors and we have no access to. The neural tube example information does not appear anywhere other than a figure in supp fig 1 that shows a 3D rendering of a surface of the neural tube, no information is provided. These details should be removed as everything is relegated to the supplementary information and there is not enough information provided to understand what was done for a reader to make any conclusions as to the validity of the outcome or applicability of the method described in the manuscript.

Author Rebuttal to Initial comments

Response to reviewers for *TubULAR: Tracking in toto deformations of dynamic tissues via constrained maps*

We thank all reviewers for their thoughtful comments, suggestions, and critiques. In our revision, we have enhanced the focus of the discussion, removing technical details where possible and clarifying areas that needed further explanation. The result is a more concise main text and greatly expanded Supplementary Information. Overall, we believe the significant revisions prompted by the discussion below have greatly enhanced the manuscript.

Below we detail the points of revision in turn in blue.

Reviewer #1:

Remarks to the Author:

Tissue cartography makes shape change in biological image data tractable in cases where the relevant dynamics occur on a tissue surface by mapping the tissue surface to two dimensional images. A limitation so far has been effective global (aka in toto) mapping of complex dynamic surfaces, with applications so far either focusing on global mapping of relatively simple static shapes like the early *Drosophila* embryo, or on local patches of complex dynamic shapes. Furthermore, computation of tensor operations on tissue surfaces had not been fully worked out, for example, gradients of rotation of vectors. Here the authors solve this problem for unbranched, simply connected surfaces of arbitrary geometry. In particular they develop a strategy to create global maps in material coordinates that minimize tissue movement in the map and implement tensor operations using discrete differential geometry.

This is a major improvement of current tissue cartography methods that should be of broad interest to readers of Nature Methods. The analysis of the shape change of the beating heart is elegant and very nicely done. However, to ensure broad impact, the manuscript would benefit from more detailed illustration of applications to morphogenesis, and the installation procedure of the tool needs to be simplified. In particular, although the authors made a very helpful website describing how to use their tool, I was not able to get either of their two main examples to run. I provide a description of my problems below.

We thank Reviewer #1 for their assessment and praise of the manuscript. We found your specific comments valuable for clarifying and enhancing the manuscript, as well as for addressing the errors you encountered while running TubULAR's example scripts.

Some specific comments:

R1.1. The main manuscript occasionally gets lost in distracting technical details. For example a description of level sets seems unnecessary. Moreover, from looking at Fig S1 it seems that the level sets generally rely on first running Ilastik segmentation and so are really just smoothing the surface of a 3D segmentation in Ilastik. That is completely fine but not stated clearly anywhere, leaving the reader to puzzle what kind of image data is needed for the level set methods to work

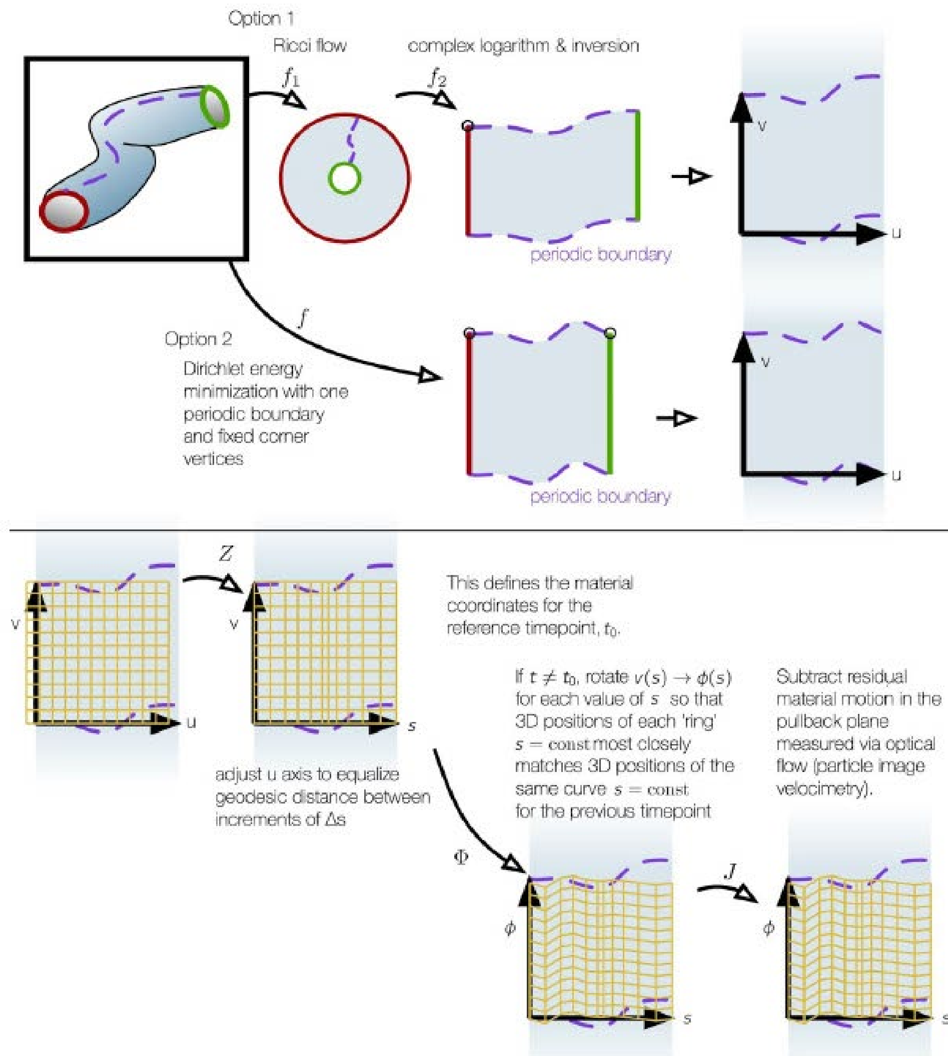
properly, because using the image as a potential/cost function directly is unlikely to work for arbitrary image data.

We appreciate this critique and have made extensive edits to the text to focus the discussion. In the revision, we have pushed most details to the SI. Among these edits, we removed all details about the level sets approach from the main text, relegating these details to the Supplementary Information. Surface detection is discussed in just a few lines before moving to the main thrust of the method.

We agree that the details of the mesh generation are not crucial to articulating the paper's main contribution. For example, smoothing a 3D segmentation would be a perfectly valid starting point for the mesh generation step of a TubULAR pipeline. We agree it is best not to overemphasize the level sets step – or any surface detection step – since this is not crucial to the main contribution. In our revision, we have pared down surface detection to just a few lines in the main text. In our revised codebase, we offer the ability of the user to simply extract the surface mesh for each timepoint as the (largest connected) isosurface of the data, which highlights the flexibility of mesh generation. We also note in the text that "[u]sers may alternately generate triangulated surfaces via other software (such as Imaris), then use TubULAR for subsequent analysis."

R1.2. In any case, surface detection was working well in previous incarnations of tissue cartography and the innovation of this manuscript lies in the definitions of the material coordinates and implementation of tensor operations. Yet the description of how material coordinates are defined conceptually could be much clearer and perhaps even deserves a main figure.

We have clarified the construction of material coordinates through edits to the section 'Constrained surface parameterization enables tracking surface dynamics in the material frame' (see PDF highlighting revisions). We also introduce the material coordinates gently in Fig 1's caption: "The surface is first mapped to the plane at a reference timepoint to define a material coordinate system." We then added a new supplementary figure reproduced below to further clarify the definition of material coordinates.



Global parameterization of tube-like surfaces with material coordinates proceeds by a sequence of mapping steps. The 3D surface is first mapped via f to the plane, either through Ricci flow (which is slower but results in a more exactly conformal map) or through minimization of a Dirichlet energy (faster but less precisely conformal, see Eq. (11). In either case, the material is periodic in the v dimension and finite in extent along the longitudinal direction u. The resulting coordinate system is then adjusted. First we apply $Z: u \to s$, where s is a geodesic distance along the longitude of the tissue defined by Eq. (15). If the timepoint under question is the reference timepoint t_0, this defines the material coordinates. Otherwise, we then apply $\Phi: v \to \phi$, where ϕ is given by Eq. (16), and then apply J to stabilize the resulting coordinates based on material motion measured through particle image velocimetry (phase correlation analysis) relative to the previous timepoint.

R1.3. Similarly, details about different conformal mapping algorithms can be moved to methods supplements.

We have followed the reviewer's suggestion and moved the details to the Supplementary Information. The main text simply states "...'unrolled' into the plane (see Supplementary Information)..." and "...where f is a conformal map…"

R1.4. It is a little disappointing that what is seemingly the main application of the tool, to make epithelial morphogenesis tractable, is left as a bit of a footnote with the main manuscript only stating: "Separating out the effects of tissue motion and cell intercalation has given insights into multiple mechanisms" and delegating an illustration to a short section at the end of the supplement which is hard to follow.

We have now ensured language throughout the text points to the main application of the tool, including:

- "Leveraging these tools has provided insight into how cell behaviors collectively drive tissue-scale and organ-scale shape change across a wide variety of systems including egg chambers, fly wings, eyes, ascidian vasculature, zebrafish endoderm, and mouse intestines. While these methods are sufficient to track tissue motion within static geometries or in local patches, *in toto* measurements of tissue deformation in complex, dynamic geometries have remained a challenge."
- "As shown in Fig 1, this provides a framework for automatically tracing the dynamics of complex shapes and facilitates cell tracking on contorting 3D surfaces. This framework then naturally decomposes tissue-frame measurements for interpretation, handling all computational subtleties that arise from the surface's curvature and bending. Performing 2D cell segmentation and projecting onto the deforming 3D surface further resolves tissue shape changes into contributions from cell shape, cell rearrangement, and cell division."
- "Figure 2D-E shows that tracked cells in the corresponding pullback images move little despite large deformations, taming the analysis of whole-organ morphogenesis. Figure S15 shows an overlay of timepoints spanning 1 hour in the material frame, highlighting the precision of pullback stabilization. Since the method rectifies tissue-scale velocity (at a user-defined scale), but not necessarily individual cells' motion, we observe cell intercalation events such as those shown in Fig. 2E. Separating out the effects of cell shape changes and cell intercalations has given insights into multiple mechanisms of morphogenesis in planar tissues (Blanchard et al 2009, Etournay et al 2015). Here, this follows naturally from our approach in organs with complex shapes. The Supplementary Information details an example of this decomposition."
- Caption for Fig 2: "...enabling quantification of cellular and tissue-scale dynamics…"

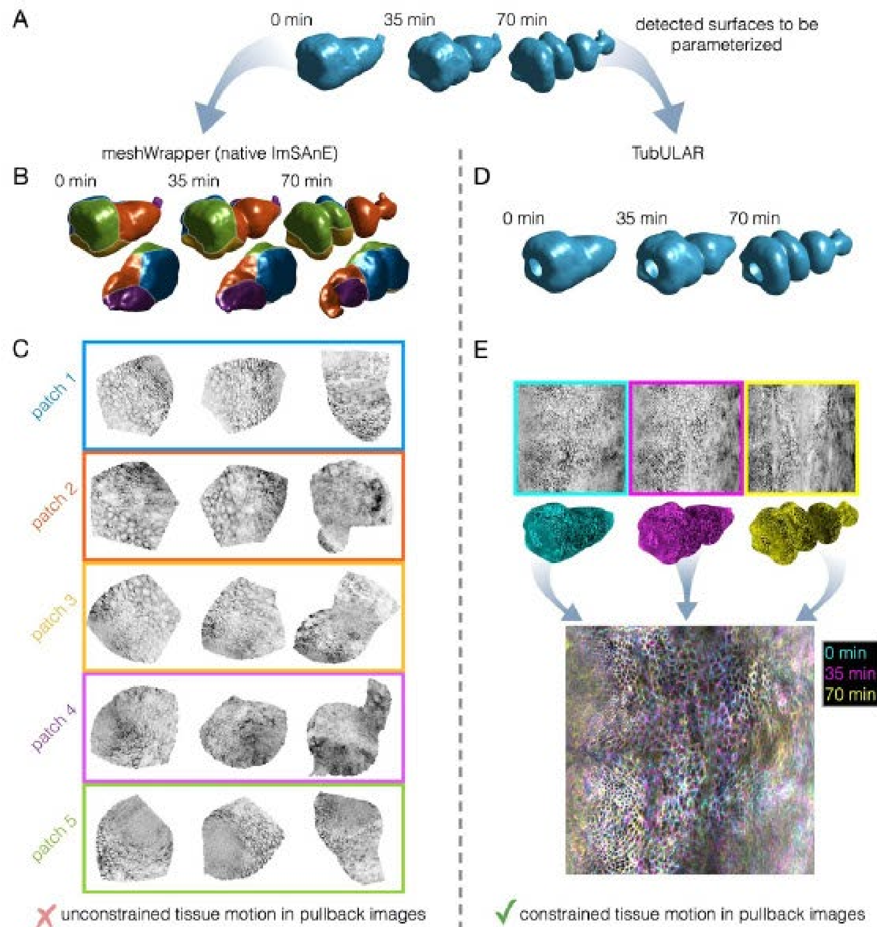
We have also edited the section at the end of the supplement, streamlining the discussion and explanations in that section.

We believe that with the other major revisions and compaction of the text, the main message will come across more strongly.

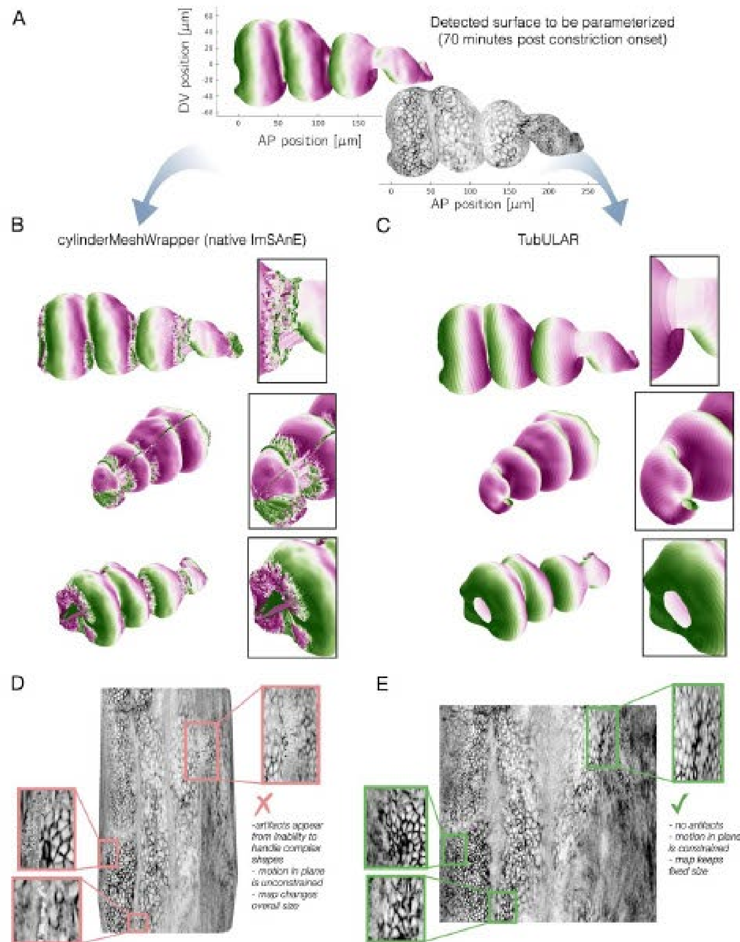
Finally, we have added several sections to the Supplementary Information that build on this message, including "TubULAR enables measurements of deforming tissue surfaces across systems" and "Validation using a synthetic dataset" (in particular the subsection "TubULAR workflow improves tracking performance"), as well as edits to "Helmholtz-Hodge decomposition of vector fields on dynamic surfaces" which speak to this point.

R1.5. The fact that there is comparison with previous methods to illustrate the improvement by tubular in the supplement is great, but it is actually not very clear that there are artifacts where the red arrows in Fig S9 are, perhaps this requires insets and/or further explanation.

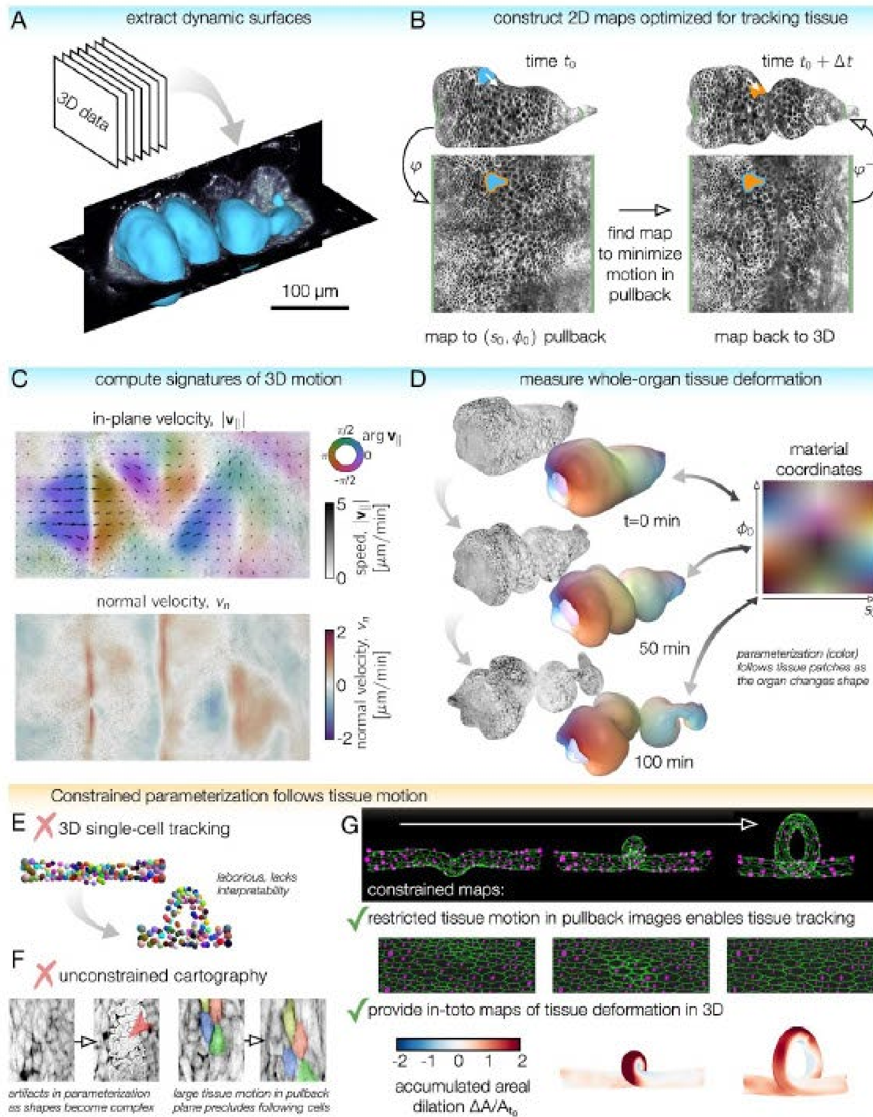
We have upgraded this figure to be two separate figures that clarify how surface fitting with meshWrapper does not adequately follow tissue motion and how cylinderMeshWrapper corrupts the 3D geometry. We likewise remade Fig 1E of the main text to clarify this point. We also discuss in the SI how pullback stabilization was not provided in ImSAnE's meshWrapper or other fitters. The modified figures are reproduced below:



Previous methods fail to follow tissue motion, here illustrated by using the meshWrapper fitter native to ImSAnE. *(A)* A detected surface results in a surface mesh triangulation to be parameterized. *(B)* Fitting the surface using ImSAnE's meshWrapper maps patches of the surface to the plane via a conformal map. *(C)* Pullback images from meshWrapper reflect large tissue motions over time, precluding cell tracking, tissue tracking, or measurements of tissue velocity. The overall image change, shape, and orientation varies from timepoint to timepoint. *(D)* In contrast, TubULAR parameterizes the full surface in a single chart, modulo two endcaps which are cut from the surface at the anterior and posterior ends to ensure a cylindrical topology. *(E)* Crucially, the resulting pullback images from TubULAR remain nearly stationary in the pullback frame due to stabilization. Here we overlay the textured surfaces (cyan, magenta, and yellow) in the material frame. The largely white cell membrane signal in the image reflects the stationary orientation of tissue in the pullback plane. Maps to the material frame from different timepoints are here taken as the fully stabilized maps $\varphi = J \circ \Phi \circ Z \circ f$.



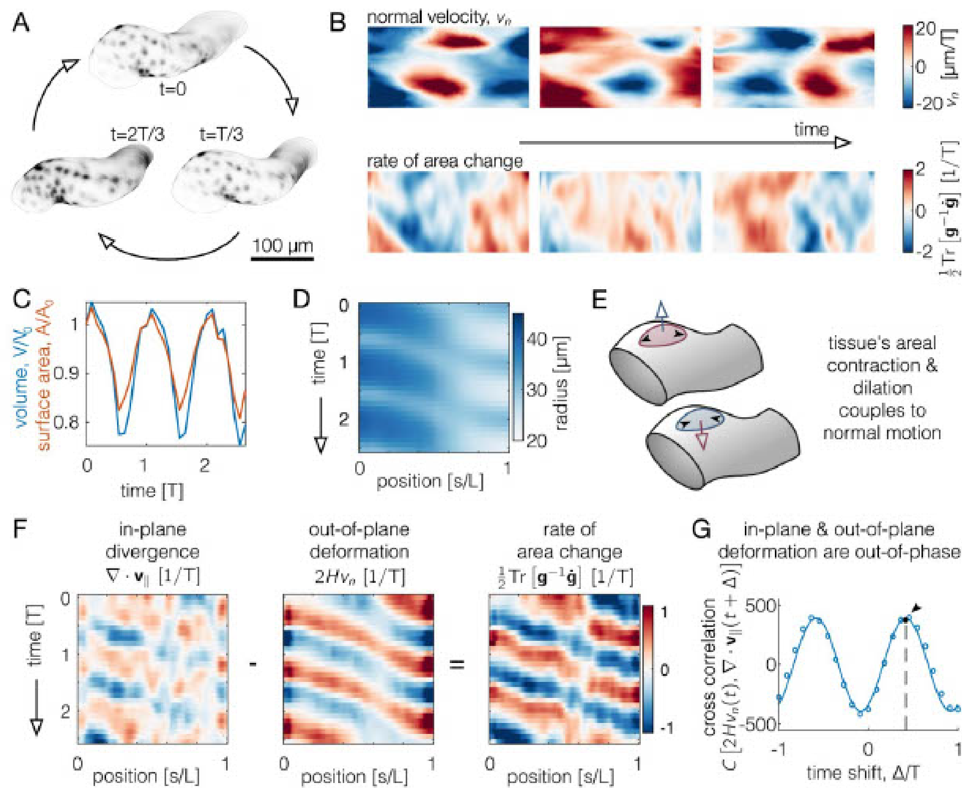
Previous methods fail to parameterize complex surface geometries, here illustrated by using the cylinderMeshWrapper fitter native to ImSAnE. *(A)* A detected surface results in a surface mesh triangulation to be parameterized by an ImSAnE fitter. *(B)* Fitting the surface using cylinderMeshWrapper results in parameterization issues because of build-in assumptions about the simplicity of the underlying cylinder-like surface. The surface is colored by the normal vector of each face projected onto the anterior-posterior axis, so that green is pointing anteriorly and purple is pointing posteriorly. *(C)* Pullback images from cylinderMeshWrapper reflect the artifacts from poor parameterization near deep folds and surface overhangs. *(D)* The parameterized surface using TubULAR preserves surface normals and preserves mesh geometry. The surface is colored as in panel (B). *(E)* The resulting pullback images from TubULAR do not have parameterization artifacts.



A
extract dynamic surfaces
3D data
100 µm
B
construct 2D maps optimized for tracking tissue
time t_0
time $t_0 + \Delta t$
φ
φ^{-1}
find map to minimize motion in pullback
map to (s_0, ϕ_0) pullback
map back to 3D
C
compute signatures of 3D motion
in-plane velocity, $|\mathbf{v}_\parallel|$
arg $\mathbf{v}_\parallel$
speed, $|\mathbf{v}_\parallel|$ [µm/min]
normal velocity, v_n
normal velocity, v_n [µm/min]
D
measure whole-organ tissue deformation
material coordinates
t=0 min
50 min
100 min
ϕ_0
s_0
parameterization (color) follows tissue patches as the organ changes shape
Constrained parameterization follows tissue motion
E
3D single-cell tracking
laborious, lacks interpretability
F
unconstrained cartography
artifacts in parameterization as shapes become complex
large tissue motion in pullback plane precludes following cells
G
constrained maps:
restricted tissue motion in pullback images enables tissue tracking
provide in-toto maps of tissue deformation in 3D
-2 -1 0 1 2
accumulated areal dilation $\Delta A/A_{t_0}$

R1.6. In fig 4B it would be nice to see the area contraction visualized in the same way as the normal velocity, perhaps it could replace the phase of the tangential velocity in the same panel.

We have followed the reviewer's suggestion and agree it improves the presentation. The figure is reproduced below:



R1.7. The math supplement is probably only readable by those that already know the math involved, and that is entirely reasonable, but it would be helpful to slightly dejargonize / help the rusty reader remember certain definitions. For example homotopically could add something like (smoothly, topology preserving), etc.

In addition to removing jargon and technical details from the main text (ex, "Helmholtz-Hodge", "Ricci flow", "dilatational", etc), we have also followed the reviewer's suggestion by extensive edits to the Supplementary Information. These edits remove jargon where possible and

introduce ideas lightly before offering further details. We also reordered some sections and promoted some subsections to sections to simplify the presentation and allow users to navigate past technical notes if desired.

In summary, this is great work that could find broad application if it were a little easier to read and use.

We thank the reviewer again for their helpful comments. We understand the reviewer also had an issue getting the environment running on their computer. To address the difficulty the reviewer experienced getting our code to run, we have included a new subsection "`Could NOT find Matlab` error while compiling gptoolbox" in the larger "Common issues in TubULAR and suggestions" page of the GitHub documentation. This provides a step-by-step solution for the problem that the reviewer faced compiling the MEX code in gptoolbox.

Reviewer #2:

Remarks to the Author:

In this work, Mitchell and Cislo present a comprehensive framework for analysing complex biological tissue shapes. They provide a powerful suite of tools that enable the user to quantify the morphological properties of tubular-like structures (of which there are many in biology). The work builds on previous tissue cartography approaches, but generalises the approach to be more robust and cope better with tissue deformations. The paper is well written and clear to follow. Further, the Supplementary Material is presented in a logical manner and approachable. The online documentation is very well curated and accessible. I tested the software for the midgut example. This worked well (though slowly on my computer). Though I don't think anyone can just use this software quickly (it's not simply a click and play software), someone with a sensible level of computing experience should be able to get the software working reasonably quickly. Originally, I was somewhat sceptical that this work represented a substantial advance on Heemskerk et al. (Nat Meth 2015). However, the combination of surface visualisation, conformal mapping and exterior calculus modules all within one framework is very powerful. Therefore, I think this work is suitable for a broad journal such as Nature Methods. However, having said that, I am not convinced it merits a full 5-figure article (discussed further below).

We thank the Reviewer for their helpful comments and praise of the work. Their critiques have prompted improvements, which we discuss below.

Major comments:

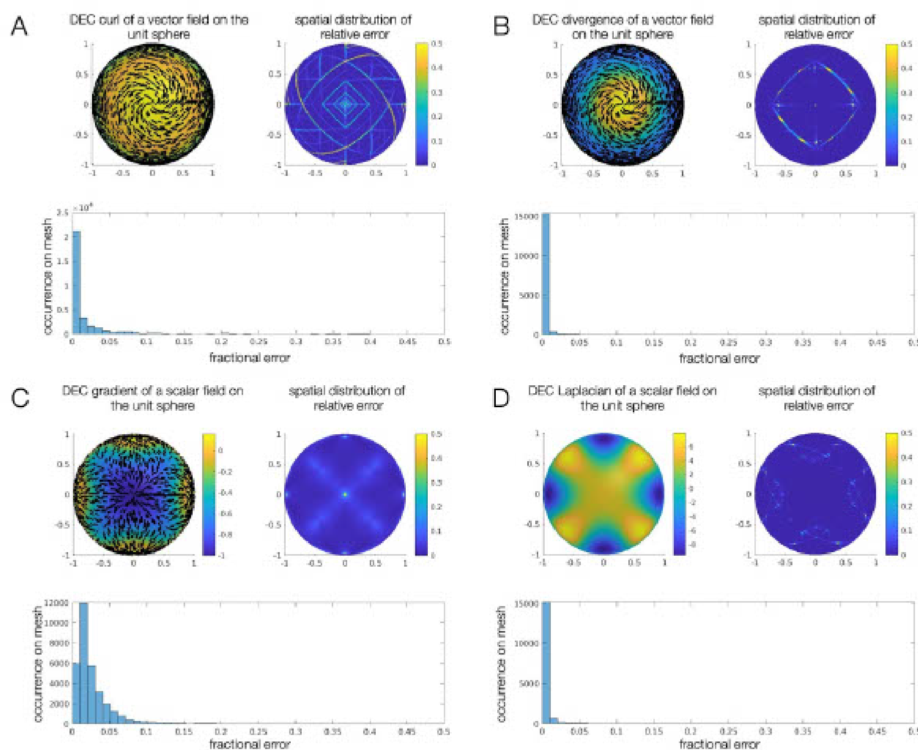
R2.1. Heemskerk and Streichan was a Brief Communication. I feel that likewise this is a more apt format for this paper. I hold this view for two reasons. First, the figures could be substantially streamlined. For example, Figures 1 and 2 are highly related and don't really merit separate representation – as they're both laying out the fundamentals behind TubULAR. Second, while the approaches outlined are very powerful in combination, each separately does not represent a major conceptual advance. The ideas here have already been developed in different contexts. The power of this work is bringing those into a coherent framework and applying to biology relevant problems; doing so requires less space than if describing new theoretical approaches. I am not fixed in this view, but better justification of the intellectual and technical advances are needed if the authors think it should stay as a longer format.

Our revised manuscript is more compact, including the abstract. We have moved several paragraphs to the SI, combined two sections, and crafted more concise explanations throughout. At the same time, some other comments requested additional information and material that we have added in the revision (ex, R2.5 and R3.1). While we are willing to pare down further and strip away figure panels, we leave this decision up to the editor. We hope that the Reviewer may find the new length agreeable.

R2.2. Looking at Figure S8, the error in the scalar field gradient appears surprisingly large, especially compared to the other measures. Can the authors provide an explanation for this and

does this impact the results (I would imagine that error over 10% could well affect the measures).

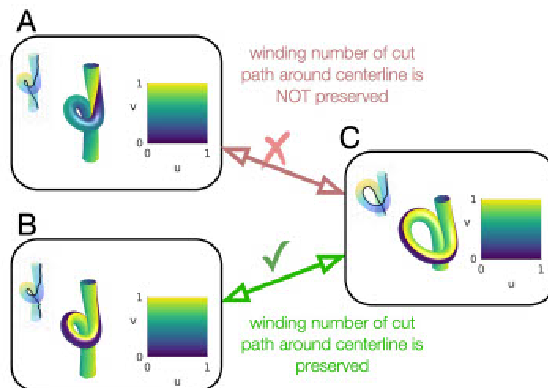
As we now describe in the SI, our DEC implementation of the scalar gradient is numerically identical to the familiar gradient operator derived from piecewise constant linear finite elements on triangle faces. This is a widely used operator (ex, see Botsch et al 2010, which is ref. [25] in the SI). The seeming discrepancy in the result stemmed from the fact that we were reporting the *fractional error*, i.e. $|\nabla f_{exact} - \nabla f_{numerical}| / |\nabla f_{exact}|$ and the original scalar function we analyzed produced a gradient with a small norm. To be precise, $\max(|\nabla f_{exact}|) \sim 0.4$ whereas $\max(|\nabla^2 f_{exact}|) \sim 10$ for the same function. In the regions where $|\nabla f_{exact}|$ was close to zero, i.e. near the north and south pole of the sphere, the fractional error was high due to the division by a small number. To avoid confusion, we have replaced the example with a different scalar function that does not exhibit this behavior. The result is reproduced below:



R2.3. As a tool, it needs to be accessible to biologists. An important point here is how "tubelike" a surface needs to be. It would be helpful to provide an example of a tissue that does not work. This will help readers to better appreciate which systems TubULAR is good for and which it isn't.

We agree that a clear understanding of the scope and limitations of TubULAR is important for readers from diverse backgrounds. To this end, we have added a new section to the SI entitled "Limitations of TubULAR". In this section, we emphasize that systems with high genus, branching topologies (e.g. branching circulatory vasculature), and systems with time-dependent topologies (e.g. systems that divide or merge) are not handled in the current implementation. We also emphasize that for analysis of systems with limited surface features (such as may result from CT scans), velocity measurements may not accurately represent the true tissue velocity since there may not be enough texture in the pullback images to resolve residual motion after constraining the parameterization.

We also discuss additional scenarios that may challenge TubULAR's ability to successfully analyze data: poor temporal resolution, extreme geometries, and incorrect winding number computation for the virtual seam. While these later issues are challenging, they may be overcome (as they were in our analysis of the *Drosophila* midgut) given appropriate parameters in TubULAR's methods. We discuss this in the SI. This new material includes the following new figure to explain a potential pitfall that the TubULAR workflow handles when assigning parameterization coordinates:

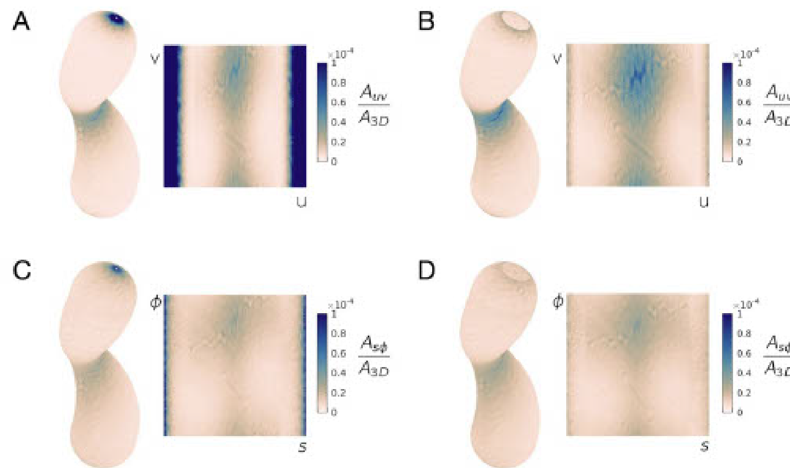


Our parameterization scheme is independent on choice of longitudinal seam (cut path) only given a topological constraint from the winding of the seam. A potential complication in a TubULAR pipeline may arise if a tube is undergoing substantial coiling. The longitudinal seam is first chosen as a geodesic connecting two endpoints (or a piecewise geodesic path for $t \neq t_0$ if complex winding of the path is detected), as shown in small insets in the top left of (A), (B), and (C). These longitudinal seams (or 'cut paths') need to exhibit a winding number about the centerline curve that matches the winding of the cut paths for adjacent timepoints. In this example for a synthetic dataset, the reference timepoint is given in (C). There, a geodesic defines the phase v around the tube in the (u,v) plane. In panel (A), a geodesic path for a different timepoint does not satisfy the same winding around the centerline. The default behavior in TubULAR is then to seek a different path that does match the topology of the adjacent timepoint. In panel (B), a piecewise geodesic path, which appears subtly different in the top left inset but winds differently around the centerline, does enable a conformal map with the same topology as that of panel (C).

R2.4. How end caps can be defined remains somewhat unclear. What are the limitations on defining such caps? Relatedly, is there a minimal size for the approach to work – e.g. could it even work on describing a single cell? I assume that at some point the discretised approximation of spatial derivatives stops being a good approximation.

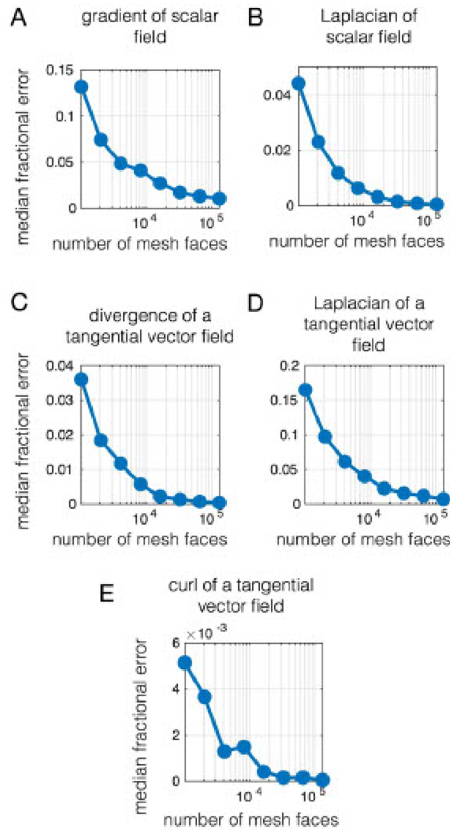
We now address both of these questions in the SI:

- ***Limits of an endcap:*** We have added an entire section entitled 'Endcap selection' and a new supplementary figure (reproduced below) to explain how this portion of the procedure functions. A single triangle of a triangulated surface is sufficient to constitute an endcap. The smaller the endcap relative to the effective radius of the rest of the sample, however, the larger the distortion in the mapped image will be.
- ***Discretization effects on DEC:*** We include an analysis of discretization effects in the DEC by comparing our discrete differential operators to analytic results on a series of triangulations of a spherical surface with increasing resolution. We have made the script generating this analysis publicly available in the DEC repository (DEC_sphericalMesh_Discretization_Error.m). The median fractional error in our results is already ≤ 5% for surfaces with ~1,000 mesh faces (a relatively coarse approximation) and decreases rapidly for more refined meshes. Our revised Supplementary Information contains these and other details.



Removing endcaps from the tube facilitates mapping to the plane, and larger endcaps can lead to smaller areal distortion in the mapping at the expense of a less complete surface parameterization. To map a tube-like object to the plane, we first remove small endcaps, defined on either end as regions within a given distance from specified endpoints. This provides two non-periodic boundaries which are mapped to $u=0$ and $u=1$. The size of the removed endcap can affect the distortion of the image in the map. This can be a problem if the distortion is so large as to affect the quality of cell segmentation or the fidelity of velocimetry measurements in the 2D pullback projections. *(A)* Removing a small portion of the tube at each end leads to large distortions near the endcaps. The distortion map is shown in color both on the 3D surface and on the 2D uv conformal parameterization. A_{uv} denotes the area of a given triangle in the discrete mesh triangulation in the uv plane, while A_{3D} is the area of the corresponding triangle in

the embedding space. *(B)* Removing a larger portion of the tube at each end leads to lower distortions near the endcaps for this example surface in the *uv* parameterization. *(C)* Similarly, in the $s\phi$ parameterization, the example surface shows largest area distortion near the endcaps. $A_{s\phi}$ denotes the area of a given triangle of the mesh in the $s\phi$ pullback parameterization. *(D)* Increasing the size of endcap likewise reduces areal distortion in the mapping.



Increasing mesh resolution improves the quality of surface derivatives calculated using DEC. We apply derivatives to scalar and vector fields defined on triangulations of the unit sphere with increasingly fine resolution (i.e. number of mesh faces). As resolution increases, median fractional error relative to analytic results diminishes. See Eq. (17) for definitions. *(A)* Error in the gradient of a scalar field relative to analytic values. *(B)* Error in the surface Laplacian of a scalar field. *(C)* Error in the divergence of a tangential vector field. *(D)* Error in the surface Laplacian of a tangential vector field. *(E)* Error in the curl of a tangential vector field.

R2.5. The implementation of DECLab and the decompositions are impressive. While it seems the code works well, it is another thing to interpret the results. Given this is for a broad readership, it will be helpful to give some specific biological examples of certain measures. For example, for the Helmholtz-Hodge decomposition, what does a "dilatational" and "harmonic" parts actually reflect in terms of the heart morphology?

Thank you for the comment. We have edited the main text discussion to remove jargon in this section. We have also added the following paragraph to the main text providing a straightforward explanation of the different velocity components calculated in the Helmholtz-Hodge decomposition:

"TubULAR further decomposes these modes to probe the signatures of motion. After separating the normal (``out-of-plane") motion from in-plane motion along the surface, the tangential component of the velocity field decomposes into three physically distinct classes of motion: dilational, rotational, and harmonic. The dilational (or ``curl-free") velocity encodes the extent to which material patches are induced to expand or contract due to in-plane motion.
The rotational (or ``divergence-free") velocity reflects swirling, vortex-like motion in which the velocity tends to circulate around a point. Finally, the harmonic component reflects surface motion that is neither contributing to in-plane expansion/contraction nor to vortex-like patterns. For tubular geometries, harmonic velocities include uniform flows along or around the tube. The application of this decomposition to the heart is shown in Fig 5A-B."

Note that we have removed the term Helmholtz-Hodge from the main text, as this adds a valence of jargon. Additionally, we have improved the corresponding sections in the SI by removing unnecessary jargon and by adding a gentle explanation of the different operators used in the DEC (and specifically in the definition of the Helmholtz-Hodge decomposition) so that less familiar readers may follow along.

Minor comments Page 3:

Typo: "… a given time point, we the point cloud …" Meaning unclear

Thank you for pointing this out. We clarified this sentence, but ultimately removed this portion of the discussion to streamline the manuscript in response to other Reviewer comments.

Drosophila should be *Drosophila* [italicised]

Thank you, this has been fixed throughout.

Is the heart really imaged in superresolution [i.e. resolution < Abbe limit]. I am not sure that's the case for the given data.

The heart was imaged with a technique dubbed '*temporal* superresolution', and was not imaged with *spatial* superresolution. This technique assigns a timestamp within a beat cycle for each

acquired frame in order to build volumes of fluorescence binned at intervals subdividing the beat cycle. This method is presented in Chan et al 2016 referenced in the main text. While we respect the cited works' choice of language about this technique, we edited the text to simply describe the approach in simple terms rather than use the term 'temporal superresolution' given the Reviewer's confusion.

Thank you again for your comments and suggestions.

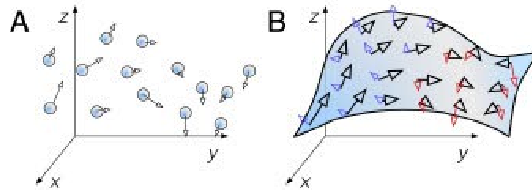
Reviewer #3:

Remarks to the Author:

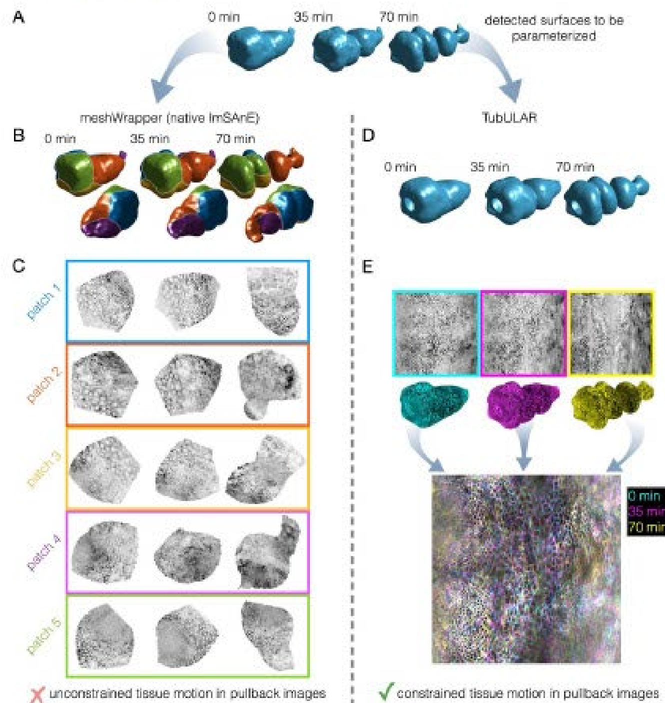
This manuscript by Drs. Mitchell and Cislo describes a methodology to capture, quantify and describe in toto measurements of tissue deformation of tubular architectures across time. They execute this through TubULAR, an open-source MATLAB framework they have developed and have freely shared. This work advances the current state of the art and integrates with the ImSAnE framework. Overall, this is a well-written manuscript, which when read in conjunction with the Supplementary Material provides detail of the approaches used and the rationale for the process chosen. The utility of this framework is high and significant, with illustrations of the applicability in two systems with dramatically different deformation and time-dependent behaviors in the developing Drosophila midgut and zebrafish heart. The fundamental challenge with this manuscript is the lack of comparative quantitative measures to determine if the solution that is generated from the method is correct/accurate. The images and pullback maps seem reasonable, and the methods and calculations performed should be correct. However, it is unclear if TubULAR provides an accurate representation of the real system or where/what cases this approach fails or larger errors are generated. Additionally, other than cartoon generalized examples comparing the approach used in the TubULAR framework relative to other methods, could there be a better quantitative description of the performance of TubULAR relative to IMSAnE for the overlapping functionality? This latter point is important as the authors specifically point out some selected failures with IMSAnE's approach as a generalized critique, but it would be beneficial for readers to understand the outcomes of the difference in error/accuracy from the different methodologies. With these details integrated, I believe this would be an important technological development that would be beneficial to the community.

We thank the reviewer for their positive comments. Thank you for prompting us to perform a more substantial quantitative evaluation of TubULAR's performance. We chose to validate the method on a synthetic dataset with extreme deformations that also challenges our workflow because of large twists in the tissue as the system coils into a loop and then uncoils into a straight cylinder. We now discuss a characterization of this synthetic dataset, with a quantitative comparison of the acquired cell positions, tracking results, and velocity fields against true values. This analysis is detailed in the new SI section 'Validation using a synthetic dataset', and the new figures are shown starting on page 21 of this response. Importantly, we find that our method accurately captures cell positions and velocities, and also greatly improves the fidelity of tracking results, even when compared to directly tracking the true cell positions in 3D space.

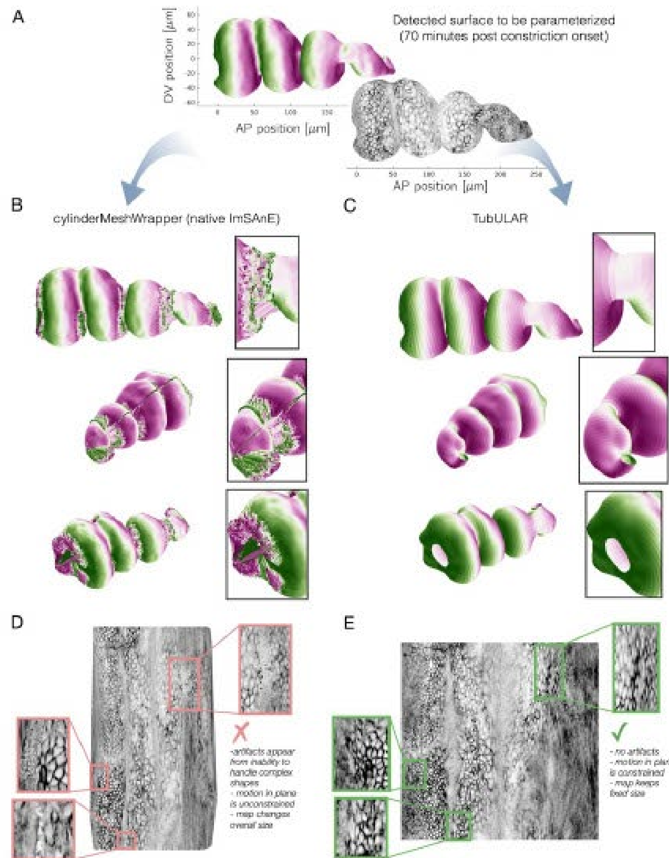
Additionally, we have included deeper comparisons of TubULAR against existing ImSAnE methods. While ImSAnE makes maps, it does not provide velocity data in 3D or decompose those 3D velocity data. Therefore, we cannot compare these aspects to ImSAnE because they were not previously implemented. We sketch this point in the image below.



With respect to static surface maps, we now include an improved comparison of TubULAR's surface parameterization methods to ImSAnE's existing methods, highlighting the artifacts that ImSAnE's naive whole-organ parameterization generates due to limitations in handling complex surface geometries (see Fig. S19 and Fig. S20 reproduced below, and see our response to Comment R1.5).



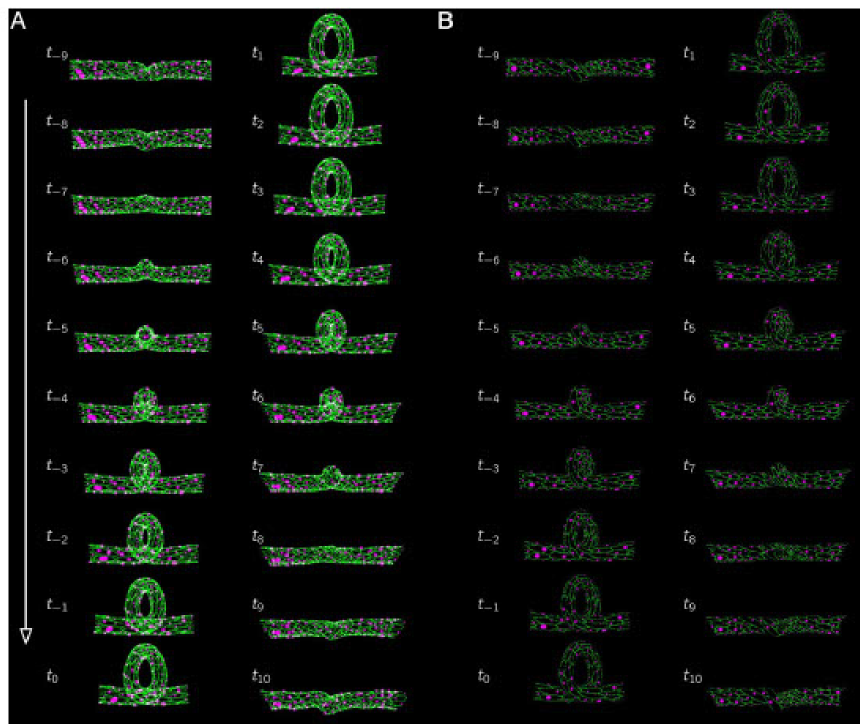
Previous methods fail to follow tissue motion, here illustrated by using the meshWrapper fitter native to ImSAnE. *(A)* A detected surface results in a surface mesh triangulation to be parameterized. *(B)* Fitting the surface using ImSAnE's meshWrapper maps patches of the surface to the plane via a conformal map. *(C)* Pullback images from meshWrapper reflect large tissue motions over time, precluding cell tracking, tissue tracking, or measurements of tissue velocity. The overall image change, shape, and orientation varies from timepoint to timepoint. *(D)* In contrast, TubULAR parameterizes the full surface in a single chart, modulo two endcaps which are cut from the surface at the anterior and posterior ends to ensure a cylindrical topology. *(E)* Crucially, the resulting pullback images from TubULAR remain nearly stationary in the pullback frame due to stabilization. Here we overlay the textured surfaces (cyan, magenta, and yellow) in the material frame. The largely white cell membrane signal in the image reflects the stationary orientation of tissue in the pullback plane. Maps to the material frame from different timepoints are here taken as the fully stabilized maps $\varphi = J \circ \Phi \circ Z \circ f$.



Previous methods fail to parameterize complex surface geometries, here illustrated by using the cylinderMeshWrapper fitter native to ImSAnE. *(A)* A detected surface results in a surface mesh triangulation to be parameterized by an ImSAnE fitter. *(B)* Fitting the surface using cylinderMeshWrapper results in parameterization issues because of build-in assumptions about the simplicity of the underlying cylinder-like surface. The surface is colored by the normal vector of each face projected onto the anterior-posterior axis, so that green is pointing anteriorly and purple is pointing posteriorly. *(C)* Pullback images from cylinderMeshWrapper reflect the artifacts from poor parameterization near deep folds and surface overhangs. *(D)* The parameterized surface using TubULAR preserves surface normals and preserves mesh geometry. The surface is colored as in panel (B). *(E)* The resulting pullback images from TubULAR do not have parameterization artifacts.

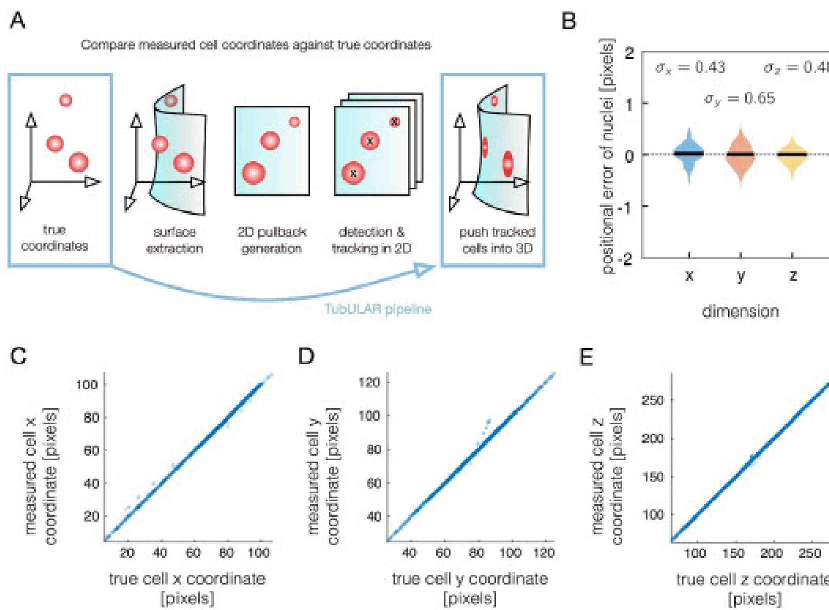
We also include a thorough quantitative analysis of the performance of our DEC implementation comparing it against analytic examples on synthetic surfaces. In particular, we have clarified the presentation of our differential operator benchmarks (see Fig. S17 and our response to Comment R2.2). We have also included a new analysis quantifying the DEC performance on surface triangulations of increasing resolution (See Fig. S18 and our response to Comment R2.4).

Finally, we also include a new SI section entitled "Limitations of TubULAR" where we describe how non-cylindrical or time-dependent topologies can cause TubULAR to fail. We also enumerate a set of scenarios where TubULAR may generate larger errors (See our response to Comment R2.3). Taken together, we believe these contributions provide a comprehensive picture of TubULAR's scope and limitations and support the conclusion that TubULAR correctly and accurately analyzes data within its realm of applicability.



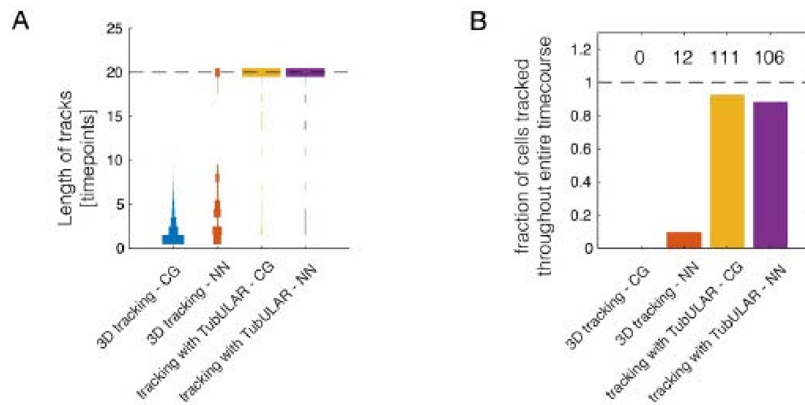
A synthetic dataset features a tube with dramatic changes in geometry, and its projection onto a surface generated in TubULAR captures the changes in shape. *(A)* The maximum intensity projection of 'nuclear' (magenta) and 'membrane' (green) channels for 20 timepoints show a tube coiling and uncoiling. Nuclear positions corresponded to a farthest-point search of 120 locations on a prescribed surface with a time-dependent centerline and a (time-dependent and space-dependent) radius around that centerline. The membrane channel was created by building a Voronoi tesselation of the nuclear positions within the prescribed surface. Nuclei are given sizes based on their distance from the nearest 'cell-cell juntion.' The prescribed surface is distinct from the

extracted surface, but the extracted surface accurately captures the prescribed surface. *(B)* The projection of the dataset onto extracted surfaces shows both the changes in shape over time and the movement of cells on the dynamic surface.



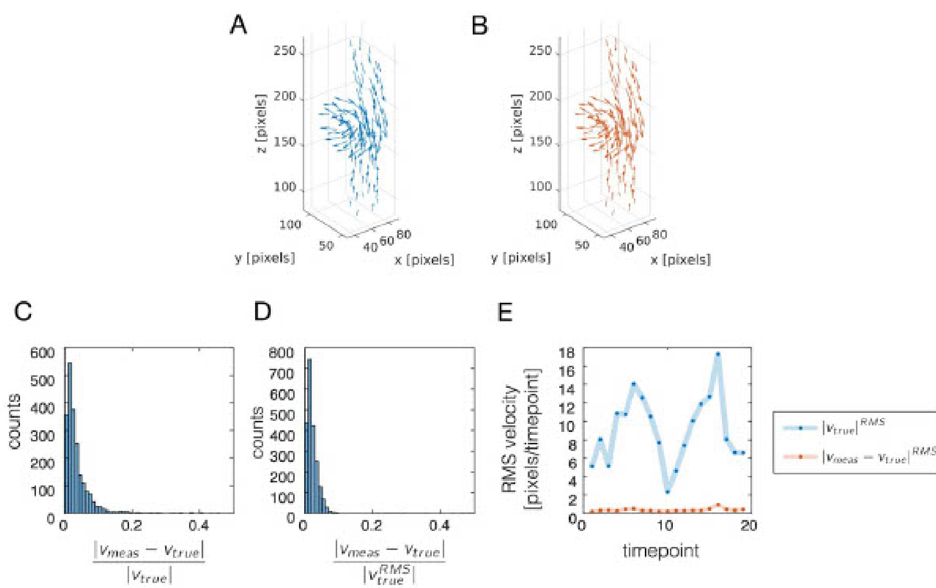
The first step of method validation compares measured nuclear positions on the extracted surface to the known input positions of nuclei in the synthetic dataset. *(A)* Schematic representation shows nuclear positions in true coordinates, which are then intersected by a detected surface. A projection to 2D captures the intersection of the data with the surface and maps those intensity values to the plane. We detect nuclei in the plane and track them across timepoints. Pushing these trajectories into 3D on the dynamic surface provides the measured cell coordinates over time. *(B)* The measured cell coordinates differ from the true (known) coordinates by a value that is typically far less than a pixel width. Median differences for x, y, and z positions are shown by a black bar. *(C-E)* Measured cell positions across tracked cells match the true input coordinates in each spatial dimension. For each plot, each blue datapoint represents a tracked cell (nucleus) position at a single timepoint, and we include positions from all timepoints.

Constrained parameterization, here shown in the $s\phi$ coordinate system, aids in tracking. Different tracking results across all 20 timepoints (colored curves) show almost no motion of nuclei despite large 3D motions of each nucleus. The tracks overlie intensity data for an example timepoint. Tracks obtained in the pullback plane via Crocker-Grier (Crocker & Grier 1996) algorithm in *(A)* and using a nearest-neighbor approach in *(B)*.



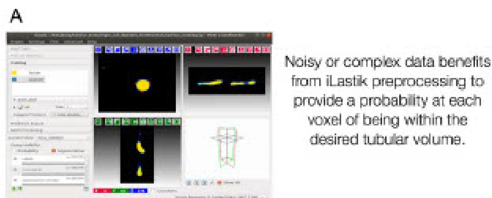
TubULAR aids automatic tracking and improves the fidelity of track trajectories. *(A)* In a synthetic dataset of 20 timepoints, few tracks (obtained by linking 3D positions of nuclei) connected objects across more than ~5 timepoints. In contrast, tracking with TubULAR resulted in most automatically-computed tracks connecting nuclei across all 20 timepoints. Dashed line denotes a track duration of 20 timepoints. *(B)* Similarly, we plot the fraction of tracks that span all 20 timepoints, highlighting that while few 3D tracks connect across the full dataset, most tracks generated in the stabilized 2D pullback images and pushed into 3D span all 20

timepoints. The total number of tracks that span all 20 timepoints is printed above each barline. The dataset had a total of 120 nuclei. 'CG' denotes 'Crocker-Grier' method for particle tracking with the largest cutoff distance permitted by the method and 'NN' denotes 'Nearest-Neighbor' method, in which each identified nucleus in time t_i is connected only with the closest identified nucleus in time t_{i+1} in 3D space (for 3D tracking) or in the 2D $s\phi$ pullback coordinate space (for TubULAR tracking).



Measured velocities using TubULAR agree with true velocities of the synthetic dataset. *(A)* An example of true cell velocities for a sample timepoint in the synthetic dataset show the difference in cell positions between the subsequent and current timepoint. *(B)* Measured cell trajectories evaluated at each tracked cell location match the true cell velocities. *(C)* The difference (normed vector difference in 3D) between measured and true cell velocities is typically less than 10\% of the true velocity magnitude. The RMS fractional error of vector differences divided by the velocity across all trajectories and all timepoints is 6%. *(D)* The difference (normed vector difference in 3D) between measured and true cell velocities is typically less than 5% of the RMS value of the true speed. The RMS fractional error of vector differences divided by the RMS velocity across all trajectories and all timepoints is 4.1%. Here $|v_{\mathrm{rms,true}}| = \sqrt{|\langle v_{\mathrm{true}} \rangle|^2}$, where the average is taken over all trajectories and all timepoints. *(E)* The RMS error in measured velocities (orange curve) is far smaller than the true RMS velocity (blue curve) for any given timepoint. Here, $|v_{\mathrm{true}}|_{\mathrm{rms}} = \sqrt{|\langle v_{\mathrm{true}} \rangle|^2}$, where the average is taken over all trajectories within a given timepoint.

A

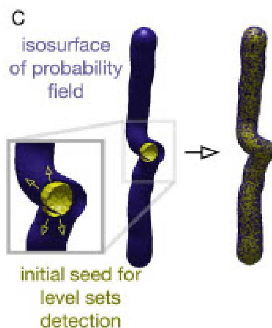


Noisy or complex data benefits from iLastik preprocessing to provide a probability at each voxel of being within the desired tubular volume.

B

The default behavior for getMeshes() is to use an active contour 'level sets' method for segmenting the volume, useful when a simple isosurface is insufficient. Select a seed point about which a level set will grow to fill a volume of similar probability values / intensities.

C



The initial level set seed (here, a sphere) evolves to fill the voxels with similar probability values / intensities, while evolving with supplied surface tension and pressure, akin to an inflating soap film. In this toy example, the probability field is already suitably smooth and precise, so the level set surface evolves to match the isosurface of the probability field.

Surface extraction using level sets aids in capturing noisy or complex geometries. *(A)* In this toy example, we segment a looped tube by first training in an iLastik workflow to create a probability field recognizing inside (yellow denotes high probability) versus outside. *(B)* In the default workflow, TubULAR generates meshes via an 'active contour' (level sets) approach. To initialize a 3D sphere to seed the growth of the estimated surface, the user can click on a location in a 2D cross-section that lies within the surface. *(C)* The resulting seed grows to fill the volume. In this toy example, the isosurface (purple) already well approximates the target surface. The initial seed (yellow sphere shown in cutaway) grows to fill the volume enclosed by the isosurface because those voxels contain similar (high) probability from the step shown in panel (A).

Additional specific suggestions to the authors to improve the manuscript:

R3.1. Description of the methods used to capture the midgut data should be provided. This was done later for the heart dataset. What is the marker that is being used to identify the midgut? The authors note that there is a monolayer of endoderm surrounded by a thin net of muscle cells. How was segmentation performed? The use of the level set method implies a discreet cell/tissue label, but it is unclear how this was done.

We have added these details to the main text:

"Throughout this section, we illustrate our method using the embryonic *Drosophila* midgut (Fig 1A-D). During embryonic development, the midgut closes into a tube composed of a monolayer

of endoderm surrounded by a thin net of muscle cells (Campos-Ortega & Hartenstein 1997, Klapper et al 2002, Reuter & Scott 1990, Beinz 1996, Wolfstetter et al 2009). Constrictions then form, subdividing the organ into chambers (Immergluck et al 1990, Mitchell et al 2022). We drive an mCherry-tagged plasma membrane marker using a midgut-specific GAL4 (Martin-Bermudo et al 1997): *w;48YGAL4;klar* x *w;UAS-mCherry.CAAX.S*. The *klarsicht* mutation in the mother reduces light scattering in the embryo, enhancing image clarity deep within the embryo at the gut surface (Welte et al, 1998)."

Further, the Supplementary Information details this procedure in the new introductory section called "TubULAR enables measurements of deforming tissue surfaces across systems". In particular, we state, "...We processed this dataset as follows: after an iLastik pass to identify the interior of the midgut tissue, TubULAR's default level sets minimization captured the apical surface of the endoderm by minimizing a Chan-Vese functional on the output of the iLastik training. The result from each timepoint was fed into the level sets optimization of the subsequent timepoint to track the tissue surface across all timepoints. We pushed this surface 2.5um outward to approximately intersect the endoderm midplane. We then mapped this dynamic surface to a 2D material parameterization and tracked cells using a semi-automated approach detailed in Mitchell et al, 2022." We additionally point the Reviewer to a new figure, Fig S10, for an illustration of this method.

R3.2. The imaging modalities this framework is compatible with should be explicitly described. The beginning of the manuscript focuses on descriptions of segmenting and constructing tissue surfaces from 3D data. While inferred from the beginning of the manuscript based on the mention of voxels, and later in the description of lightsheet imaging for the zebrafish heart dataset, explicit discussion of compatible image data architectures would be valuable to the reader. i.e. this method cannot be directly applied to confocal z-stacks, 2D data to obtain 3D information, pixel structure vs voxels.

This method is perfectly compatible with confocal z-stacks, and we have emphasized this point in the main text. We have further clarified that this method is compatible only with 3D voxelated data.

Two points in which this is clarified are:
"Contemporary microscopy methods such as confocal microscopy (Pawley 2006) or light-sheet microscopy (Keller et al 2008, Krzic et al 2012, Chen et al 2014) generate volumetric data, wherein each voxel carries a (potentially multi-channel) intensity measured at a specific location in the sample."

and

"... segmentation of the tissue surface from 3D data -- whether from confocal, light-sheet, or another 3D imaging technique,..."

We note that some 3D imaging techniques such as (micro) CT scans may not provide sufficient 'texture' (variation in intensity along the surface) to track in-plane deformations, in which case velocity measurements may not be faithful. The toolkit may nonetheless be useful for such data. We discuss this more in a new section of the SI titled 'Limitations of TubULAR.'

R3.3. At the end of the manuscript, there are claims of documented examples of this process having been used for neural tube and tracking a phase-separated droplet in a microtubule gel. Minimal information and no detail on method was described in the supplementary info for the latter (figure ref label is also incorrect), with methods referring to a paper that has been "submitted" by a group that is not the authors and we have no access to. The neural tube example information does not appear anywhere other than a figure in supp fig 1 that shows a 3D rendering of a surface of the neural tube, no information is provided. These details should be removed as everything is relegated to the supplementary information and there is not enough information provided to understand what was done for a reader to make any conclusions as to the validity of the outcome or applicability of the method described in the manuscript.

We have removed references to these systems from the main text and instead simply cite those papers that used some portion of methods presented, as reproduced below. The citation for Tayar et al 2022 has been updated, as it is now public.
"An efficient method for tracing surface dynamics in the Lagrangian frame of reference offers new opportunities for understanding not only organ dynamics during morphogenesis, but also organoids, *in vitro* systems, and sub-cellular structures (Karzbrun et al 2021, Tayar et al 2022, Lemma et al 2022)."

We added a section to the SI to discuss each panel of Figure S1 in more detail. This section is titled 'TubULAR enables measurements of deforming tissue surfaces across systems'.

Thank you again for your comments and suggestions.

Decision Letter, first revision:

Dear Noah,

Thank you for submitting your revised manuscript "TubULAR: Tracking in toto deformations of dynamic tissues via constrained maps" (NMETH-A49947B). It has now been seen by the original referees and their comments are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Methods, pending minor revisions to satisfy the referees' final requests and to comply with our editorial and formatting guidelines.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. Please do not upload the final materials and make any revisions until you receive this additional information from us.

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https://www.springernature.com/gp/researchers/orcid/orcid-for-nature-research

Thank you again for your interest in Nature Methods. Please do not hesitate to contact me if you have any questions. We will be in touch again soon.

Sincerely,
Madhura

Madhura Mukhopadhyay, PhD
Senior Editor
Nature Methods

Reviewer #1 (Remarks to the Author):

The edited manuscript is much improved. Although I think it could be streamlined further, I am satisfied with the changes.
(As an example for further streamlining: “In toto imaging of the heart relied on light-sheet illumina- tion of a transgenic Tg(cmlc2:eGFP) embryo expressing GFP in cardiomyocytes [56]. These data were taken us- ing an approach which combines frames from multiple heartbeat cycles to build volumetric data at 11 equally- spaced phases of the heart beat cycle [35]. “ is distracting and could be left out with ref to source of the data or mentioned in the figure caption.)
My main remaining concern is that I am still not able to run the code to test it. With the updated troubleshooting instructions the Matlab not found error was resolved. However, now cmake runs into library linking errors copied below that I do not have time to resolve. I do think the will end up being used much more broadly if either much more detailed instructions or some kind of precompiled version that runs out of the box could be provided (at least in my department the vast majority of people use Macs with a recent version of MacOSX).
While I hope the authors will take this advice to heart I think the work could be published without further delay.

Compiling the C compiler identification source file "CMakeCCompilerId.c" failed.
Compiler: /Library/Developer/CommandLineTools/usr/bin/cc
Build flags:
Id flags:

The output was:
1

ld: library not found for -lSystem
clang: error: linker command failed with exit code 1 (use -v to see invocation)

Compiling the CXX compiler identification source file "CMakeCXXCompilerId.cpp" failed.
Compiler: /Library/Developer/CommandLineTools/usr/bin/c++
Build flags:
Id flags:

The output was:
1
ld: library not found for -lc++
clang: error: linker command failed with exit code 1 (use -v to see invocation)

Reviewer #2 (Remarks to the Author):

The authors have done a very good job of addressing my concerns. I have no specific comments.

Author Rebuttal, first revision:

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Response #2 to reviewers for *TubULAR: Tracking in toto deformations of dynamic tissues via constrained maps*

We thank the reviewers for their assessment.

Reviewer #1:
Remarks to the Author:

The edited manuscript is much improved. Although I think it could be streamlined further, I am satisfied with the changes.
(As an example for further streamlining: "In toto imaging of the heart relied on light-sheet illumina- tion of a transgenic Tg(cmlc2:eGFP) embryo expressing GFP in cardiomyocytes [56]. These data were taken us- ing an approach which combines frames from multiple heartbeat cycles to build volumetric data at 11 equally- spaced phases of the heart beat cycle [35]. " is distracting and could be left out with ref to source of the data or mentioned in the figure caption.)

Thank you for the encouragement to streamline further. We have shortened the section in question, as well as other sections. Our revised manuscript now has a Methods section. We have pushed some technical details that were previously in the main text to the Methods section, including details of fly and fish strains.

My main remaining concern is that I am still not able to run the code to test it. With the updated troubleshooting instructions the Matlab not found error was resolved. However, now cmake runs into library linking errors copied below that I do not have time to resolve. I do think the will end up being used much more broadly if either much more detailed instructions or some kind of precompiled version that runs out of the box could be provided (at least in my department the vast majority of people use Macs with a recent version of MacOSX).
While I hope the authors will take this advice to heart I think the work could be published without further delay.

We appreciate this feedback and addressed this issue. Our modifications to TubULAR during our latest revision have removed all dependencies requiring compilation, with the sole exception being the 'toolbox_fast_marching' software used during the centerline computation. This should solve the issue you were facing. Our edits limit dependencies to a minimum.

In our revision, we made substantial edits to the documentation to make the tool more accessible. Among these, we have moved all discussion of optional dependencies to a later section of the documentation. We also made extensive edits to make the tool compatible with Windows operating systems.

Thank you for your library linking errors. This software can be compiled directly from within MATLAB using a supplied script without the use of CMake or the terminal. We have successfully tested the compilation of this software from within MATLAB on Linux, MacOS, and Windows.

We have also updated our documentation with explicit instructions for this minimal compilation step.

While the compilation step is no longer needed for core functionality, we comment briefly on your specific errors here in case you want to explore the additional functionality. The output message suggests that the linker command was unable to find the libraries named “libSystem” and “libc++”. Unfortunately, the error messages do not provide a complete context for the observed problem and it is difficult to diagnose it without fully understanding the environment on your MacOSX system.

Some likely issues/troubleshooting strategies include:

(1) A missing or corrupted library
    - Ensure the libraries “libSystem” and “libc++” are available somewhere on your system.
(2) An incorrectly set library path
    - Verify that the library paths are correctly set to include the location of the libraries in question by checking the “LIBRARY_PATH” environment variable.
(3) Improperly installed/out-of-date Xcode Command Line Tools
    - Make sure that these tools are installed by running ‘xcode-select - -install’ in Terminal.
(4) An invalid CMake configuration
    - Double-check that the CMake configuration is correctly pointing to the compiler and the libraries you intend to use.
(5) A corrupted CMake cache
    - Try deleting the CMake cache files and then re-run the configuration step.

Reviewer #2:
Remarks to the Author:

The authors have done a very good job of addressing my concerns. I have no specific comments.

We thank Reviewer #2 for their assessment of the manuscript and their earlier suggestions.

Final Decision Letter:

Dear Noah,

I am pleased to inform you that your Article, "TubULAR: Tracking <i>in toto</i> deformations of dynamic tissues via constrained maps", has now been accepted for publication in Nature Methods. Your paper is tentatively scheduled for publication in our December print issue, and will be published online prior to that. The received and accepted dates will be 05 Sep, 2022 and 10 Oct, 2023. This note is intended to let you know what to expect from us over the next month or so, and to let you know where to address any further questions.

Acceptance is conditional on the data in the manuscript not being published elsewhere, or announced in the print or electronic media, until the embargo/publication date. These restrictions are not intended to deter you from presenting your data at academic meetings and conferences, but any enquiries from the media about papers not yet scheduled for publication should be referred to us.

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Please feel free to contact me if you have questions about any of these points.

Best regards,
Madhura

Madhura Mukhopadhyay, PhD
Senior Editor
Nature Methods