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COMMENTARY

Accuracy in Quantitative 3D Image Analysis

George W. Bassel[1]

School of Biosciences, University of Birmingham, Birmingham B15 2TT, United Kingdom

Quantitative 3D imaging is becoming an increasingly popular and powerful approach to investigate plant growth and development. With the increased use of 3D image analysis, standards to ensure the accuracy and reproducibility of these data are required. This commentary highlights how image acquisition and postprocessing can introduce artifacts into 3D image data and proposes steps to increase both the accuracy and reproducibility of these analyses. It is intended to aid researchers entering the field of 3D image processing of plant cells and tissues and to help general readers in understanding and evaluating such data.

Advances in digital imaging have led to the generation of an increasing number of 3D data sets (Truernit et al., 2008; Fernandez et al., 2010; Kierzkowski et al., 2012; Roeder et al., 2012). Whole-mount and time-lapse imaging enable all cells in an organ to be analyzed in 3D over time, providing a comprehensive analysis of plant growth and development (Roeder et al., 2011).

The generation of these 3D image data sets has led to the development of novel computational approaches to facilitate their analysis (Cunha et al., 2010; Kierzkowski et al., 2012; Bassel et al., 2014; Yoshida et al., 2014). With the development of these new methods comes a need for quality control and standard measures to ensure the accurate analysis of data sets. An overall objective of this approach is the accurate capture and quantification of the 3D geometry of biological objects. The inaccurate abstraction of shape data and introduction of artifacts during image acquisition and postprocessing must be kept to a minimum.

Following imaging, typically using confocal microscopy, 3D objects can be identified through the process of segmentation (Roeder et al., 2012). This can be achieved using automatic seeding through a watershed approach or by inflating "balloons" with defined seeds in individual cells (Federici et al., 2012). Vertices and meshes that describe cell surfaces may then be generated using an algorithm such as marching cubes (Lorensen and Cline, 1987). Vertices at defined spacings can be placed on the surface of unique segments, and the surfaces describing these geometric shapes are represented by the triangles connecting adjacent vertices constituting a polygonal mesh.

The mesh describing segment surfaces is ultimately what defines the shape of an object in question. Rough and irregular features are often represented by meshes owing to the imperfect nature of data collection from biological samples (Desbrun et al., 1999; Taubin, 2000). In the context of plant cells whose surfaces are naturally smooth, the segmentations and meshes describing them are in practice noisy and contain undesirable geometric irregularities. The lower the quality of the original image being segmented, the greater the irregularities in the mesh that describe the shape.

In order to improve the quality of irregularly triangulated polygonal meshes, smoothing operations can be performed. In this way, the roughness of the surface can be reduced, improving the texture and representation of a segmented object.

A straightforward and easy to implement operation to remove noise in 3D meshes is Laplacian smoothing (Field, 1988). This process repositions vertices to an average position (barycentre) along a mesh surface to create a smoothed effect. However, Laplacian smoothing has the side effect of slightly shrinking the object in question (Taubin, 2000). While this shrinkage effect is well documented among computer scientists who develop algorithms to modify polygonal meshes, it is perhaps less well understood and discussed by the end user biological community.

Smoothing of meshes has the positive effect of making surfaces smoother and removing noise, while enhancing the visual aesthetic of segmented objects providing the appearance that their geometry has been captured accurately (Figures 1A and 1B). In cases where objects have been poorly segmented, the need to remove the jagged appearance of meshes is greater, and additional smoothing steps are often used. This repeated Laplacian smoothing leads to additional smoothing-induced shrinkage and greater abstraction of the object being analyzed. Given that the mesh itself is intended to represent the 3D geometry of an object in question, changing its overall size by smoothing represents a perturbation and manipulation of data that inaccurately reflects the quantitative capture of geometry. The need to remove rough edges in meshes due to noise needs to be balanced with the accuracy that the mesh represents an object in question.

An example of an unsmoothed mesh representing the cells of 3D segmented plant organ can in seen in Figure 1A. The rough edges and irregularities of this primary unsmoothed mesh, coming from a suboptimal noisy confocal image stack, do not accurately represent the surface of these plant cells. The mesh in Figure 1B, which has been smoothed once, appears to be a more accurate representation of cell shape than the unsmoothed mesh in Figure 1A and has not been shrunk dramatically.

In the context of plant organ cellular segmentations, additional Laplacian smoothing steps create even smoother cells, but

[1]Address correspondence to g.w.bassel@bham.ac.uk.

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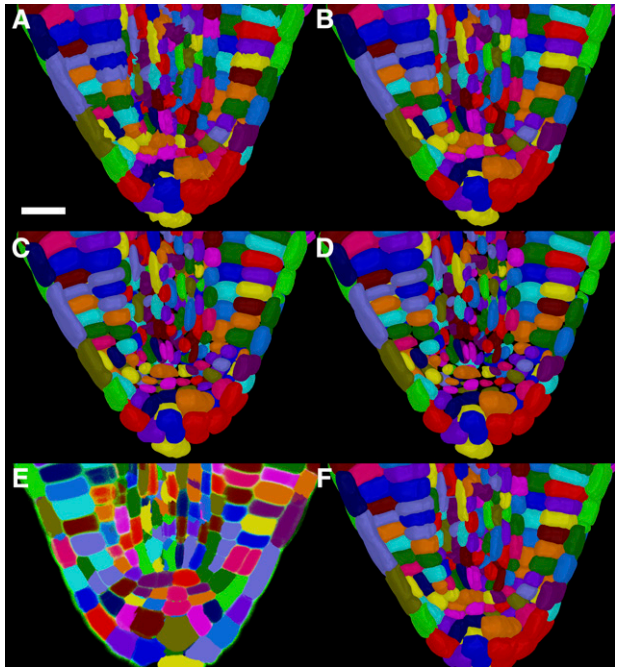

Figure 1. Effect of Laplacian Smoothing on the Cellular Structure of a 3D Segmented Arabidopsis Radicle.

(A) Surface rendering of a mesh following generation using marching cubes with a cube size of 2 μm and no smoothing. Bar = 10 μm.
(B) Same as **(A)** following one Laplacian smoothing pass.
(C) Same as **(A)** following six smoothing passes.
(D) Same as **(A)** following nine smoothing passes.
(E) An original confocal stack showing cell walls in green and the multicolored segmented stack before generating the mesh using marching cubes.
(F) Smoothing of the mesh in **(A)** using the Taubin λ/μ algorithm with λ = 0.5, μ = −0.53, and nine smoothing steps.

also exaggerated gaps between adjacent cells due to cell shrinkage (Figures 1C and 1D). These spaces do not reflect reality as adjacent cells are physically appressed against their cell walls, which are rarely more than several microns thick. This example demonstrates the abstraction of cell shape that can occur following multiple Laplacian smoothing steps, and the gaps between cells are a hallmark of data that have been postprocessed to the point of inaccuracy.

Other factors can affect the response of 3D segmented cells to Laplacian smoothing. These include mesh triangle size, with larger triangles being more susceptible to shrinkage (Desbrun et al., 1999), and cell size, with smaller cells being more susceptible to smoothing-based shrinking than larger cells (Figure 1D).

If the purpose of 3D segmentation is strictly qualitative, smoothing-based shrinkage may not present a problem. However, if quantitative analyses are applied to shrunken meshes, this will result in inaccurate data.

MOVING FORWARD WITH ACCURACY IN QUANTITATIVE 3D IMAGE ANALYSIS

Ways in which accuracy, transparency, and reproducibility in 3D image analysis can be improved are proposed below.

A first objective in the accurate analysis of 3D objects is enhanced primary image quality. The original stack is the closest representation of the true 3D geometry of an object of interest, and segmentations from high-quality confocal stacks often do not require smoothing due to the low noise present. Minimizing the postprocessing of both the image and subsequent mesh should therefore be a priority in maintaining quantitative image analysis standards.

Improving Initial Sample Materials

For the purpose of edge detection and segmentation, a high signal-to-noise ratio of object boundary to surrounding area is required (Moreno et al., 2006; Cunha et al., 2012; Roeder et al., 2012). This can be achieved by adjusting primary image acquisition settings to have near saturation on object boundaries and an absence of signal in adjacent regions. Fuzzy and incomplete edges make accurate and consistent boundary detection a challenge, while oversaturation during image acquisition can lead to poor segmentation.

A higher signal-to-noise ratio may also be achieved using stronger cell boundary markers (Geldner et al., 2009; Cunha et al., 2012), improving sample preparation and clearing (Truernit et al., 2008), and using more sensitive detectors on confocal microscopes (Roeder et al., 2012). Alternative image acquisition strategies may also be used such as multiangle reconstruction (Fernandez et al., 2010). This allows for the greater capture of cell walls than is possible with single-angle acquisition, especially in the z-direction where resolution is limiting.

Some biological materials are better suited for live imaging and 3D segmentation than others. Limitations in image contrast, resolution, blurring, and noise level lead to poor signal-to-noise ratios for edge detection. Challenging samples to image include optically heterogeneous specimens, for example, the mature plant embryo that has highly refractive cell walls and oil bodies causing the spherical aberration of light (Moreno et al., 2006; Bassel et al., 2014).

Primary Image Acquisition

Image stack acquisition can be improved for the purposes of segmentation by closing the pinhole on the confocal to reduce optical

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slice size, while also decreasing the step interval between adjacent slices in a z-stack. This has a positive effect on the ability to accurately segment cell shape without further postprocessing manipulations (Cunha et al., 2012).

Segmentation of Images

A commonly used algorithm to segment cells is the 3D watershed algorithm (www.itk.org). This approach can lead to artifacts including irregular cell shapes and artificial outgrowths in segmented cells that tempt the use of smoothing to enhance their appearance. The development of methods to correct these segmentation errors, for example, using a machine learning-based approached to recognize irregular shaped cells, will help reduce the need for the manipulation of meshes through, for example, smoothing.

Detailed Description of Image and Mesh Postprocessing

A clear description of the methods and steps taken to generate and analyze 3D data sets is essential. As many of these methods are still evolving and under development, consistency and transparency in the technical description are paramount.

Key methodological information includes (1) details of sample preparation, (2) image acquisition settings, (3) image preprocessing steps, (4) the triangle size used to generate polygonal meshes, and (5) the type of smoothing and the number of smoothing passes that were performed on meshes. The combination of triangle size, cell size, and number of Laplacian smoothing passes will influence the accuracy with which meshes properly capture 3D objects, making this critical to the interpretation of their geometry.

The inclusion of original stacks together overlayed with segmented images in supplemental information will also provide an indication as to the quality of the segmentations being presented. In this way, the relationship between the segmentation and the original biological sample can be directly compared. This is shown in the context of the example in Figure 1E, allowing the size of the cells in the mesh in Figures 1A to 1D to be compared with the original and segmented stacks.

Alternative Mesh Smoothing Operations

Smoothing procedures that avoid shrinkage of meshes have been developed (reviewed in Taubin, 2000) and represent alternative options that maintain the geometric integrity of meshes following smoothing operations. For example, the λ/μ algorithm follows Laplacian smoothing (λ) with an "unshrinking" step using the negative scaling factor μ (Taubin, 1995). Application of this approach to the example in Figure 1 had a positive effect on reducing mesh shrinkage and may represent an alternative to Laplacian smoothing (Figure 1F). A quantitative assessment of this and other methods in response to diverse mesh types is warranted (Morigi et al., 2012; Gargallo-Peiro et al., 2014).

Quantification of Segmented Images Rather Than Meshes for Cell Volumes

Prior to the generation of meshes is the process of segmentation where unique objects are identified. It is possible to perform some analyses, such as cell volume calculations by counting image voxels, directly using these segmented images (Fernandez et al., 2010; Bassel et al., 2014). In the case of volumetric measurements, this may be a favorable approach to analyzing cell size when segmentations are performed accurately on high-quality confocal stacks.

While the direct analysis of segmented image stacks can prevent errors due to post image processing, the accuracy of this approach is difficult to assess due to visualization limitations. At the same time, to date, rough edges on segmented stacks cannot be enhanced by smoothing processes. Biological images are inherently noisy, and the segmentation of cell surface edges invariably is not smooth. A certain degree of smoothing, in particular using surface-preserving algorithms, removes this noise and provides more accurate geometric representations of 3D objects. For example, the measurement of linear cell anisotropy on segmented cells with highly irregular surfaces would lack accuracy if performed directly on a stack. These irregularities could be reduced following mild surface-preserving smoothing on their corresponding meshes.

Objective Observation of Segmented Objects

Objective and critical analysis of segmented objects remains important in the generation, review, and critical analysis of this digital data type. The presentation of objects that qualitatively do not resemble the structure they claim to be describing should be interpreted as inaccurate data.

CONCLUSION

Quantitative 3D imaging is both exciting and visually impressive and provides meaningful insight into plant development. Quality control of these data sets is key to ensuring that acquired data accurately represent the biology and that the information derived using this approach is of rigorous scientific quality. Stochastic computational elements in segmentation and mesh creation processes mean that identical results cannot be obtained with each iteration of image analysis; however, the steps outlined here should help to increase the accuracy and reproducibility with which these analyses are performed and help with the evaluation of these data by the scientific community. The suggestions in this commentary may not be the only options to improve the accuracy of quantitative 3D image analysis, and the rapid developments in this field will continue to provide additional solutions. Further discussion that addresses biologically relevant issues of accuracy and reproducibility will help ensure scientific rigor is maintained while using these techniques.

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REFERENCES

Bassel, G.W., Stamm, P., Mosca, G., Barbier de Reuille, P., Gibbs, D.J., Winter, R., Janka, A., Holdsworth, M.J., and Smith, R.S. (2014). Mechanical constraints imposed by 3D cellular geometry and arrangement modulate growth patterns in the Arabidopsis embryo. Proc. Natl. Acad. Sci. USA **111:** 8685–8690.

Cunha, A., Tarr, P.T., Roeder, A.H.K., Altinok, A., Mjolsness, E., and Meyerowitz, E.M. (2012). Computational analysis of live cell images of the *Arabidopsis thaliana* plant. Methods Cell Biol. **110:** 285–323.

Cunha, A.L., Roeder, A.H.K., and Meyerowitz, E.M. (2010). Segmenting the Sepal and Shoot Apical Meristem of *Arabidopsis thaliana*. IEEE Engineering in Medicine and Biology, pp. 5338–5342.

Desbrun, M., Meyer, M., Schroder, P., and Barr, A.H. (1999). Implicit fairing of irregular meshes using diffusion and curvature flow. SIGGRAPH '99 Conference Proceedings, pp. 317–324.

Federici, F., Dupuy, L., Laplaze, L., Heisler, M., and Haseloff, J. (2012). Integrated genetic and computation methods for in planta cytometry. Nat. Methods **9:** 483–485.

Fernandez, R., Das, P., Mirabet, V., Moscardi, E., Traas, J., Verdeil, J.L., Malandain, G., and Godin, C. (2010). Imaging plant growth in 4D: robust tissue reconstruction and lineaging at cell resolution. Nat. Methods **7:** 547–553.

Field, D.A. (1988). Laplacian smoothing and Delaunay triangulations. Commun. Appl. Numer. Methods **4:** 709–712.

Gargallo-Peiro, A., Roca, X., and Sarrate, J. (2014). A surface mesh smoothing and untangling method independent of the CAD parameterization. Comput. Mech. **53:** 587–609.

Geldner, N., Dénervaud-Tendon, V., Hyman, D.L., Mayer, U., Stierhof, Y.D., and Chory, J. (2009). Rapid, combinatorial analysis of membrane compartments in intact plants with a multicolor marker set. Plant J. **59:** 169–178.

Kierzkowski, D., Nakayama, N., Routier-Kierzkowska, A.L., Weber, A., Bayer, E., Schorderet, M., Reinhardt, D., Kuhlemeier, C., and Smith, R.S. (2012). Elastic domains regulate growth and organogenesis in the plant shoot apical meristem. Science **335:** 1096–1099.

Lorensen, W.E., and Cline, H.E. (1987). Marching cubes: A high resolution 3D surface construction algorithm. In SIGGRAPH '87 Proceedings of the 14th Annual Conference on Computer Graphics and Interactive Techniques, pp. 163–169.

Moreno, N., Bougourd, S., Haseloff, J., and Feijo, J. (2006). Imaging Plant Cells. (New York: Springer Science and Business Media).

Morigi, S., Rucci, M., and Sgallari, F. (2012). Nonlocal surface fairing. Lect. Notes Comput. Sci. **6667:** 38–49.

Roeder, A.H.K., Cunha, A., Burl, M.C., and Meyerowitz, E.M. (2012). A computational image analysis glossary for biologists. Development **139:** 3071–3080.

Roeder, A.H.K., Tarr, P.T., Tobin, C., Zhang, X., Chickarmane, V., Cunha, A., and Meyerowitz, E.M. (2011). Computational morphodynamics of plants: integrating development over space and time. Nat. Rev. Mol. Cell Biol. **12:** 265–273.

Taubin, G. (1995). A signal processing approach to fair surface design. In SIGGRAPH '95 Conference Proceedings, pp. 351–358.

Taubin, G. (2000). Geometric signal processing on polygonal meshes. In EUROGRAPHICS 2000.

Truernit, E., Bauby, H., Dubreucq, B., Grandjean, O., Runions, J., Barthélémy, J., and Palauqui, J.C. (2008). High-resolution whole-mount imaging of three-dimensional tissue organization and gene expression enables the study of phloem development and structure in Arabidopsis. Plant Cell **20:** 1494–1503.

Yoshida, S., Barbier de Reuille, P., Lane, B., Bassel, G.W., Prusinkiewicz, P., Smith, R.S., and Weijers, D. (2014). Genetic control of plant development by overriding a geometric division rule. Dev. Cell **29:** 75–87.