Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Plant Biology

Quantifying morphogenesis in plants in 4D

George W Bassel[1] and Richard S Smith[2]

Plant development occurs in 3D space over time (4D). Recent advances in image acquisition and computational analysis are now enabling development to be visualized and quantified in its entirety at the cellular level. The simultaneous quantification of reporter abundance and 3D cell shape change enables links between signaling processes and organ morphogenesis to be accomplished organ-wide and at single cell resolution. Current work to integrate this quantitative 3D image data with computational models is enabling causal relationships between gene expression and organ morphogenesis to be uncovered. Further technical advances in imaging and image analysis will enable this approach to be applied to a greater diversity of plant organs and will become a key tool to address many questions in plant development.

Addresses
[1] School of Biosciences, University of Birmingham, Birmingham B15 2TT, UK
[2] Department of Comparative Development and Genetics, Max Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, 50829 Cologne, Germany

Corresponding author: Smith, Richard S (smith@mpipz.mpg.de)

Current Opinion in Plant Biology 2016, **29**:87–94

This review comes from a themed issue on **Growth and development**

Edited by **Doris Wagner** and **Dolf Weijers**

For a complete overview see the Issue and the Editorial

Available online 1st January 2016

http://dx.doi.org/10.1016/j.pbi.2015.09.008

1369-5266/© 2015 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

In order to understand how genes control geometry and the emergence of form [1], it is necessary to relate shape change to gene expression patterns. Often the relationships are complex, and simulation modeling has emerged as an important tool to aid the biologist [2••,3,4••,5], in a discipline termed *computational morphodynamics* [6–8].

This area is being driven in part by the development of new microscopy techniques that are enabling the collection of cell geometry and gene expression at increasingly higher resolutions in both space and time [9–12]. In step with advances in image acquisition hardware, new and increasingly powerful software specifically targeted at plants is being developed to quantify cell shape and gene expression at the cellular and subcellular levels [13••,14••,15•,16–18].

Many studies have looked at development in 2D, as developmental events can often be abstracted to 2D layers of cells [19–22]. However the full 3D phenotype is the developmental output of the genome, and quantifying this in its complete form is becoming a primary objective of developmental genetics and biology. Although many recent advances in 3D plant imaging have also been made at the whole plant and whole organ scale [23–30], here we will focus on cellular level analyses.

Fixed samples versus live imaging

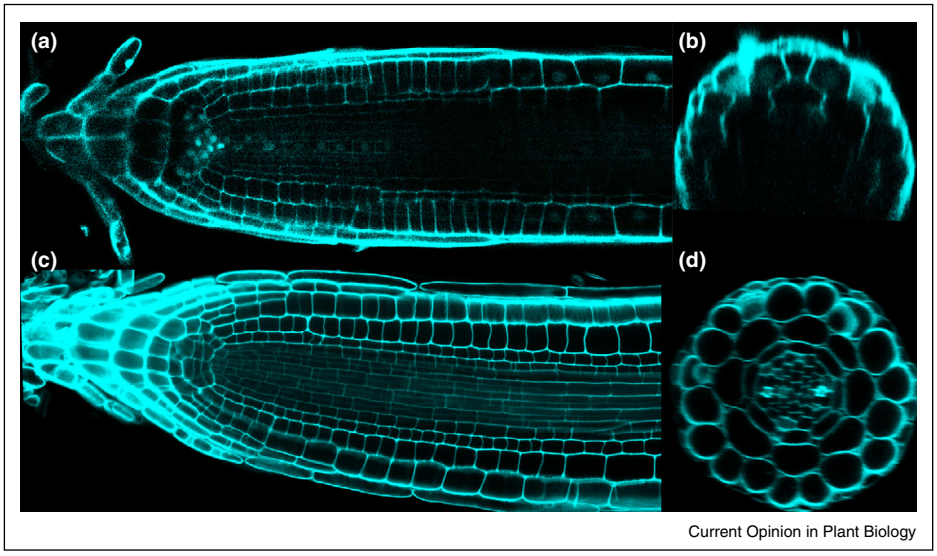
A common way to image plant cells in 3D is to use conventional confocal laser scanning microscopy which generates a *z*-stack of 2D (*x*–*y*) optical sections through the sample [31]. Imaging can be performed using either live or fixed tissue, and the two methods have their own advantages and disadvantages.

Live imaging requires *in planta* staining technique using a vital fluorescent marker, such as green fluorescent protein (GFP), which can be targeted to the cell wall, membrane or nucleus, depending on the type of analysis being performed. Several marker lines have been specifically developed to label geometry in living plant cells [32,33••]. Cell walls may also be fluorescently stained with vital dyes including propidium iodide (PI) or FM4-64. A key advantage to a live imaging approach is the ability to observe the same cells over time, however there is a significant loss of fluorescent signal as one images deeper into tissues (Figure 1).

An alternative is to use fixed tissue that is stained and then cleared using a mounting media such as chloral hydrate, which has a refractive index similar to glass [31] (compare Figure 1a,b with Figure 1c,d). Although this destroys vital fluorescence markers such as GFP, gene expression can still be monitored by using GUS reporter lines and imaging the reflection of the crystals which form as the product of the enzymatic reaction using 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid as a substrate [34]. The use of fixed tissue provides vastly superior resolution and the ability to image deep into plant tissues, and as a result yields superior 3D geometry for quantitative analysis. The key disadvantage to this approach is that only static images can be analyzed, complicating the analysis of the dynamics of cellular processes.

Nonetheless, plant organs which consistently ordered cellular patterning, including roots, embryos, and hypocotyls,

Figure 1



Comparison of live and fixed tissue imaging in the mature *Arabidopsis* root. **(a and b)** Live images mature root stained with propidium iodide in longitudinal and cross section. **(c and d)** Mature root fixed, stained and clear using the mPA-PI method [34].

can be imaged using fixed tissue and dynamic developmental series reconstructed using single cell digital atlases [2••,4••,35•].

In the case of both live and fixed plant tissue, 3D images taken with confocal microscopy have much lower resolution in the z-direction, and the signal from cell walls perpendicular to the view angle can be very low. To address this issue, Fernandez *et al.* [13••] proposed a method whereby samples are imaged from several different angles and the stacks merged to create a single stack.

Image acquisition

Advances in confocal microscopy are both enhancing z-stack quality for 3D image analysis and creating new opportunities where limitations existed previously. Current confocal microscopes are now available with a new generation of high sensitivity detectors, enabling weak signals deep within plant organs to be detected. The use of two photon confocal microscopy can enhance the retrieval of fluorescence signal deeper within live imaged tissue and fixed samples alike [36], and is less photo-toxic. Selective Plane Illumination Microscopy (SPIM or light sheet microscopy), has also been applied to live image plant organs in both primary and lateral roots [37,38]. Selective plane illumination also reduces photo-toxicity, and although the resolution for a single stack is not as good as with conventional single-photon confocal, combining data from multiple angles (stack fusion) provides an option to obtain superior 3D images with this method [38].

Other emerging technologies for 3D imaging of fixed samples rely on ultra-thin sectioning techniques. This greatly increases z resolution as sections can be down to the tens of nanometers, and signal strength does not depend on depth within the tissue. Serial block face SEM [39,40] progressively removes sections from the sample block, and the remaining block face is imaged using SEM. Since the block is imaged rather than the section, this method largely avoids the distortion introduced from cutting, and associated registration difficulties. Compared to normal sectioning which can be immunolabeled, this approach is more limited in its capacity to image gene expression as SEM compatible gene reporter constructs are required [41]. High resolution X-ray computed tomography (HRXCT) has also shown promise to achieve cellular resolution in fixed samples [42•], although in principle live imaging is possible if appropriate contrast enhancing agents are developed.

Cellular versus nuclear 3D analyses

Cellular level 3D analyses of plant organs can be divided into two broad approaches. In the simpler of the two, only the plant nuclei are computationally extracted from images. The geometric properties of the cells from which these nuclei come, including their size and shape, must be inferred from the spacing of the nuclei [15•]. A disadvantage is that in larger cells, anisotropic cell shape and nuclear movement can influence the accuracy of these calculations.

Nuclear analysis is well suited to performing live imaging in plant organs [37,38]. Unlike cell shape calculations,

tracking nuclei over time gives an accurate representation of cell division rates [15•,43] and cell lineages [44]. Nuclear analyses are also particularly useful in the quantification of single cell gene expression where ratiometric analysis of reporter fluorescence can be performed against a constitutively expressed control [33••,45•].

A more precise, and more demanding alternative to nuclear analyses is to segment the cell walls fully in 3D to extract complete cell geometry [2••,4••,13••,31,46,47]. This requires the cell boundary to be fully labelled using a fluorescent protein targeted to the plasma membrane marker line, or the use of a cell wall and membrane stains such a propidium iodide or FM4-64. Cell wall stains have the advantage that they can be used on species where genetic tools are not available, however can have issues with toxicity and uneven staining. It is also straightforward to separate PI signal from YFP or GFP reporter lines, enabling the collection of both geometry and gene expression simultaneously on different channels.

The nuclei versus cell wall approaches are not mutually exclusive. Geometry can be extracted with using a cell wall stain, and gene expression from a nuclear signal [38,43]. It is even possible to use a nuclear marker to generate seeds for a segmentation algorithm designed to extract the cell geometry from a cell wall signal collected on another channel [33••]. In practice the main limitations are the number of colors that can be reliably separated, which in plants is often limited to around three.

3D image segmentation

Following the collection of 3D image stacks, computational analyses are required to extract either the nuclei and/or cell walls to provide quantitative information about the cells. In order to accomplish this, segmentation algorithms are used to divide the image into objects of interest. This can be done at the tissue level, to extract the global shape of an organ, or at the cellular-level and even the subcellular levels when segmenting nuclei or subcellular compartments. In order to have an accurate cell segmentation, a high signal to noise ratio is required [2••,4••,48–50], and in most cases the simplest way to improve segmentation results is to refine collection techniques. The next step is to try to improve the quality of the raw images with filters such as edge-preserving anisotropic diffusion [15•], or edge detection and enhancement [46].

Once the images are pre-processed, a segmentation algorithm is performed. A common method used for this is the watershed algorithm. In this algorithm the image signal intensity is viewed like the height in terrain (in 2D). Seeds for regions are placed in the valleys (lowest signal pixels), and then the pixels are added to the regions in order of their intensity. The regions grow until they meet, segmenting the image into cells. Often some sort of region merging method is applied afterward, to fuse over-segmented cells. It is usually easier to process an over segmented image by fusing regions, than it is to split cells in an under segmented image. The manual editing and correction of segmented images in 3D remains a challenge.

In order to improve on results obtained with the watershed algorithm, several methods have been used that attempt to incorporate a priori information about the image. Plant cells are fairly restricted in the types of shapes they can take. For example, with the watershed, it is very common for two cells to become joined because there is a small hole in the signal at one of the walls. To overcome this, Federici *et al.* [33••] have used a balloon segmentation algorithm. A small virtual balloon is placed inside the cell and inflated until it hits the high signal area comprising the cell boundary. There is an energy cost for bending so that the balloon will not protrude into a neighboring cell through a small hole.

Another approach to tackling this problem includes computationally improving holes in cell walls before segmentation [46]. Binarization of weak cell wall signal using direction-selective local thresholding enabled the creation of a complete boundary which reduced cell fusion following segmentation. Machine learning methods may also hold promise for improving 3D segmentation results in plant cells [51].

The output of 3D cell segmentation is usually in the form of another 3D image, where voxel values represent labels for the cells they belong to. Simple analyses of cell shape, including surface area and volume, may be done directly using these images [13••]. However often it is convenient to convert the segmented bitmap image into a polygonal mesh using an algorithm such as 3D marching cubes [52]. This facilitates shape analysis and visualization of the cells [4••,35•,47,48,53].

2.5D analysis

The technical limitations of imaging opaque plant tissue suggest that full 3D live-imaging of plant tissue will remain difficult in many tissues. However many patterning processes in plant development occur in or near the epidermal layer of cells [19,54,55]. Plant cells are encased within shared cell walls that physically adhere cells to their neighbors, so they do not move with respect to each other during growth. In many plant organs, this symplastic growth allows the determination of the principle growth rate in all cells from analyzing the surface [56]. Similarly, in thin flat structures such as leaves, many interesting developmental decisions can be examined by tracking the surface deformation [57–60], or the events happening in only a few cells deep in the tissue [61]. This suggests the possibility to simplify many problems to 2D. Typically, 2D images are obtained from a 3D stack by projecting it

(i.e. maximum intensity) onto a plane, or by taking an optical section (which may be oblique) through the sample. One can also combine the two, such as a thick optical section [22]. However the key problem is that the resulting image is flat, compressing information into plane and losing the 3D shape of the structure.

To overcome this problem, it is possible to work directly with the curved surface itself, a structure that contains 3D shape information at the organ level, and is accessible with current live-imaging technologies. Dumais and Kwiatkowska (2002) [62] developed the sequential replica technique [63] where surface molds of the shoot meristem were taken over several time points allowing the creation of resin replicas which could be imaged by SEM. Imaging each cast at several angles allows the surface to be reconstructed in 3D, and has been used to track growth in the shoot apex [64••] (Figure 2a).

Barbier de Reuille *et al.* [14••] have developed a method to extract the organ surface and generate a curved surface image (2.5D) directly from 3D confocal stacks (Figure 2b). By projecting the signal normal to the surface an undistorted image of the surface cell shape is created, eliminating the artifacts from a 2D slice or projection. The method has been used to track growth, gene expression, and cell division on the surface in a variety of plant organs [56,57,65•] (Figure 2c). The combined 2D-3D nature of surface projections also makes it straightforward to extract the principle directions of growth [62], facilitating studies where the anisotropy of growth plays an important role. Organ level analysis, such as surface curvature and global shape, is also possible with these methods [56].

Cell type recognition

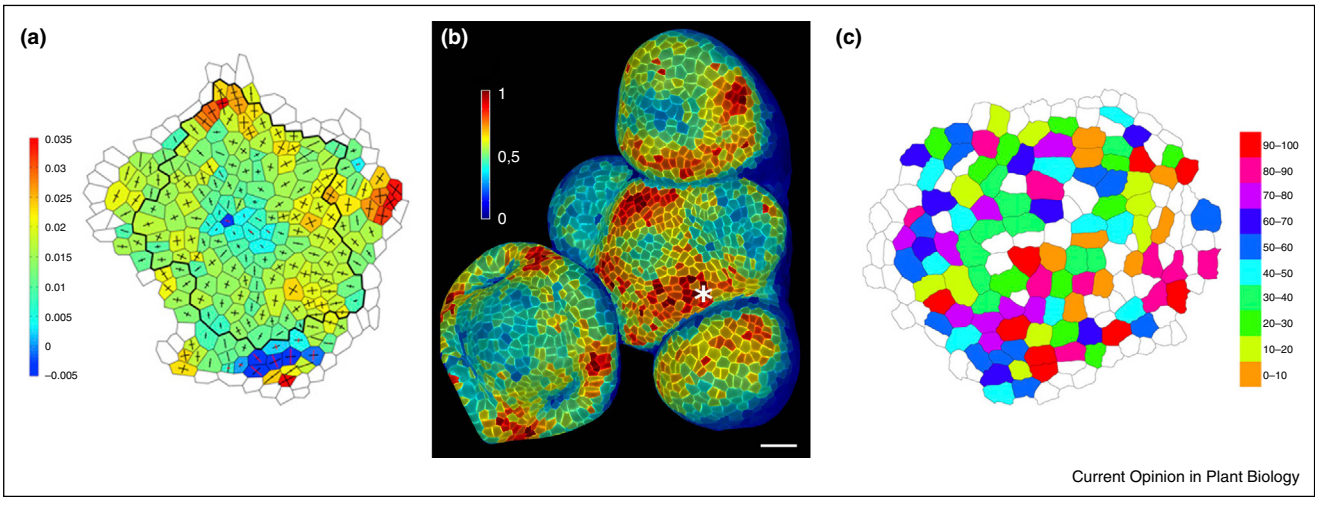
Image datasets, like gene expression datasets, require annotation in order to extract relevant local information within organs. Since 3D imaging is able to capture many more cells of multiple cell types deep within plant organs, there is an increased need to be able to identify and annotate cells in order to extract meaningful information from these images.

This has been done manually in 3D [4••], or automatically using the intrinsic 3D cell geometry of cells in radially symmetric organs [35•] (Figure 3c,d). 3D automatic annotation has also been achieved for nuclei in roots [15•] (Figure 3b). Both of the latter approaches make use of the natural local co-ordinate system radially symmetric plants organs possess [66] (Figure 3a). However, there remains a need to develop tools to annotate 3D images of organs with diverse and non-symmetric cellular architectures.

The 4th dimension

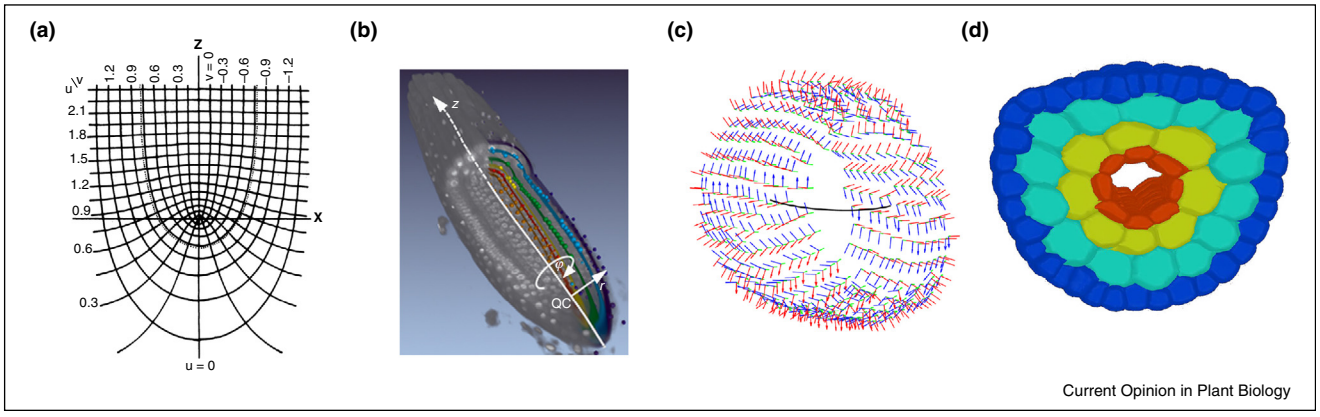
In order to quantify changes in cell shape or gene expression during plant development, it is necessary to identify and track equivalent cells over time. This can be done by live-imaging cells over multiple time points and then finding the correspondence between them. On larger samples landmarks have been identified in consecutive images at the super-cellular level, and the correspondence made between the time points [58,59].

Figure 2



Cellular level analysis on 2.5D surfaces of the *Arabidopsis* shoot apex. **(a)** Growth analysis in the *katanin* mutant of in an *Arabidopsis* inflorescence meristem over 24 h using the replica method and SEM imaging [64••]. Lines represent growth rates along principal directions with black positive and red negative. Cell color represents areal growth rate. **(b)** Cellular-level quantification of the protein reporter construct PIN1-GFP in the epidermal layer of the shoot apex using 3D confocal images [14••]. Color bar in arbitrary units of intensity. **(c)** Comparison of cell division patterns to Erera's rule in a shoot apex extracted from 3D confocal images [65•]. Colors represent bins by percentile with orange representing the best fits, and red the worst.

Figure 3



Applying a natural coordinate system for a plant organ. **(a)** Hejnowicz and Karczewski. [66] propose a natural coordinate system for the root to interpret growth and cell division. **(b)** Schmidt *et al.* [15•] and **(c and d)** Montenegro-Johnson *et al.* [35•] use a similar method to aid cell annotation and the calculation of growth rates in organ-centric terms. (b) Alignment of the co-ordinate system using the nuclei of the root. (c) Placement of a central Bezier curve through the centre of a hypocotyl, and alignment of 3 axes within each cell representing the principal directions. (d) Hypocotyl false colored by cell type.

A more precise method is to segment the images into cells, and to use the cells themselves as landmarks [13••,14••,56,57,64••]. Assigning the lineage from parent to daughter cells is often done manually, however Fernandez *et al.* [13••] propose a graph-matching method that seeks to find the most likely cell lineage automatically [13••]. Another approach seeks to find the deformation field directly between the two original images by performing a non-rigid registration of the images. This method used by Fernandez *et al.* [13••] to find an initial correspondence, which is then improved with their graph-based method.

When using fixed samples, there is the advantage of increased accuracy and capture of cellular architecture, however it is not possible to do time lapse analysis on the same organ to track cells. In certain cases it has been possible to extract growth data from fixed samples. For example, using the non-dividing cortical cells in the *Arabidopsis* embryo as an internal cellular indexing system, cell positions were defined and tracked enabling equivalent cells to be compared across samples [2••,35•]. The method was used to reconstruct the dynamic series of cell expansion events driving seed germination and hypocotyl elongation using static images taken over time.

3D cellular patterning

Cell division patterns are particular importantly in plants where cells are unable to move, unlike their animal counterparts. In addition, patterning events can be influenced by the shape of the cell itself, for example in pavement cells [67], or vascular initials [68•]. As cell geometry is intimately related to cell fate in plants, the study of 3D cell geometry and division patterns becomes of central importance. An example of this is the 3D volumetric analysis of division patterns in the early developing *Arabidopsis* embryo that revealed the presence of asymmetric divisions that were previously thought to be symmetric [4••]. The involvement of an auxin-mediated mechanism regulating these asymmetric cell divisions was revealed through the analysis of mutant embryos. A refinement of division rules in the shoot meristem has also been accurately defined through the quantification division planes in 2.5D [65•].

Gene and protein expression analysis in 3D images

In order to understand how regulatory networks control multicellular morphogenesis, both 3D cell geometry gene and expression and/or protein abundance must be able to be quantified simultaneously. This can be achieved in 3D by looking at the total amount of reporter present within the volume of an individual cell captured by a mesh [35•,69], or in 2.5D by projecting the amount of signal contained in a volume onto a curved surface representing that cell [22,56]. Having quantitative information on 3D cell shape and quantitative gene expression enables the relationship between signaling networks and cellular dynamics to be uncovered. The quantitative nature of this information lends it to statistical analysis linking the relationship between regulatory network and cell shape dynamics [35•].

Combining 3D data with modeling

The value of 3D morphometric data is greatly enhanced when integrated into a model which has a targeted hypothesis. A key feature of growth models is to understand the cellular, genetic, and signaling interactions in

the context of 3D growth of plant organs [70]. As plant growth is a mechanical process, the mechanical interactions between cells and their feedbacks is an integral part of multicellular morphogenesis [2••,71–73]. This has spurred a trend towards mechanical simulation on more realistic 3D templates of plant organs at cellular resolution [2••,73].

In order to yield valid results with numerical approaches such as the finite element method (FEM), there is a need to generate FEM-friendly cellular meshes, where the aspect ratios of the elements are not too large. It is more efficient and convenient for simulation if nodes on shared walls are not duplicated. Typical iso-surface extraction techniques such as 3D marching cubes have not been optimized to extract such conjoined cellular structures. As a result, meshes derived directly from segmented 3D images are not optimal for mechanical simulation. A targeted computational approach to generating mechanically appropriate meshes is therefore required [74•]. The paucity of tools available to generate optimal 3D meshes from segmented images has constrained most work to highly simplified meshes instead [2••,73,75–77].

3D imaging into the future

3D image analysis and modeling in plants is in its infancy and holds great promise to uncover further mechanistic principles underlying plant growth and development. Central to advances in the area will be improvements in 3D image acquisition at cellular resolution, and additional computational tools to analyze these digital data. Limitations in live imaging and recovering signal deep within tissues remains a central obstacle. The continued development of novel imaging and software makes 3D analysis in plants a growing and exciting field ready to pursue a greater diversity of biological questions.

Acknowledgements

We thank Petra Stamm for the images used in Figure 1. G.W.B. was funded by BBSRC Grants BB/L010232/1 and BB/J017604/1 and R.S.S. was funded by the Max Planck Institute for Plant Breeding Research, Cologne. We thank Miltos Tsiantis for helpful discussions.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Coen E, Rolland-Lagan AG, Matthews M, Bangham JA, Prusinkiewicz P: **The genetics of geometry**. *Proc Natl Acad Sci U S A* 2004, **101**:4728-4735.

2. •• Bassel GW, Stamm P, Mosca G, de Reuille PB, Gibbs DJ, Winter R, Janka A, Holdsworth MJ, Smith RS: **Mechanical constraints imposed by 3D cellular geometry and arrangement modulate growth patterns in the *Arabidopsis* embryo**. *Proc Natl Acad Sci U S A* 2014, **111**:8685-8690.
Changes in 3D cell geometry during *Arabidopsis* embryo growth are extracted from confocal images using non-time lapse, fixed samples. Data are integrated into mechanical 3D simulations to uncover the regulation of growth by a combination of mechanical and genetic inputs.

3. Sauret-Gueto S, Schiessl K, Bangham A, Sablowski R, Coen E: **JAGGED controls *Arabidopsis* petal growth and shape by interacting with a divergent polarity field**. *PLoS Biol* 2013:11.

4. •• Yoshida S, de Reuille PB, Lane B, Bassel GW, Prusinkiewicz P, Smith RS, Weijers D: **Genetic control of plant development by overriding a geometric division rule**. *Dev Cell* 2014, **29**:75-87.
3D image analysis is used to identify cell division patterns during *Arabidopsis* embryogenesis. Integration of data into computational models led to novel insights into the role of auxin in cellular patterning.

5. Yadav RK, Perales M, Gruel J, Girke T, Jonsson H, Reddy GV: **WUSCHEL protein movement mediates stem cell homeostasis in the *Arabidopsis* shoot apex**. *Genes Dev* 2011, **25**:2025-2030.

6. Roeder AHK, Tarr PT, Tobin C, Zhang XL, Chickarmane V, Cunha A, Meyerowitz EM: **Computational morphodynamics of plants: integrating development over space and time**. *Nat Rev Mol Cell Biol* 2011, **12**:265-273.

7. Chickarmane V, Roeder AHK, Tarr PT, Cunha A, Tobin C, Meyerowitz EM: **Computational morphodynamics: a modeling framework to understand plant growth**. *Annu Rev Plant Biol* 2010, **61(61)**:65-87.

8. Jonsson H, Gruel J, Krupinski P, Troein C: **On evaluating models in computational morphodynamics**. *Curr Opin Plant Biol* 2012, **15**:103-110.

9. Truong TV, Supatto W, Koos DS, Choi JM, Fraser SE: **Deep and fast live imaging with two-photon scanned light-sheet microscopy**. *Nat Methods* 2011, **8** 757-U102.

10. Helmchen F, Denk W: **Deep tissue two-photon microscopy**. *Nat Methods* 2005, **2**:932-940.

11. Huang B, Wang WQ, Bates M, Zhuang XW: **Three-dimensional super-resolution imaging by stochastic optical reconstruction microscopy**. *Science* 2008, **319**:810-813.

12. Dupuy L, Mackenzie J, Haseloff J: **Coordination of plant cell division and expansion in a simple morphogenetic system**. *Proc Natl Acad Sci U S A* 2010, **107**:2711-2716.

13. •• Fernandez R, Das P, Mirabet V, Moscardi E, Traas J, Verdeil JL, Malandain G, Godin C: **Imaging plant growth in 4D: robust tissue reconstruction and lineaging at cell resolution**. *Nat Methods* 2010, **7**:547-553.
The authors use image collection from multiple angles to generate a single stack with improved resolution. They then segment such images over multiple time points and track the lineage of cells automatically in 3D.

14. •• Barbier de Reuille P, Routier-Kierzkowska AL, Kierzkowski D, Bassel GW, Schupbach T, Tauriello G, Bajpai N, Strauss S, Weber A, Kiss A *et al.*: **MorphoGraphX: a platform for quantifying morphogenesis in 4D**. *Elife* 2015, **4**:05864.
Software is presented to analyze 4D image datasets. The software is unique in that it enables image processing, cell segmentation and lineage tracking on curved '2.5D' images. This makes it especially well suited to work with confocal image stacks from plant samples.

15. • Schmidt T, Pasternak T, Liu K, Blein T, Aubry-Hivet D, Dovzhenko A, Duerr J, Teale W, Ditengou FA, Burkhardt H *et al.*: **The iRoCS toolbox — 3D analysis of the plant root apical meristem at cellular resolution**. *Plant J* 2014, **77**:806-814.
A cylindrical coordinate system is used to aid the identification and annotation of cells in the root apex. The method enables the extraction of cell types and geometric parameters from 3D confocal stacks.

16. Peng HC, Ruan ZC, Long FH, Simpson JH, Myers EW: **V3D enables real-time 3D visualization and quantitative analysis of large-scale biological image data sets**. *Nat Biotechnol* 2010, **28** 348-U375.

17. Boudaoud A, Burian A, Borowska-Wykret D, Uyttewaal M, Wrzalik R, Kwiatkowska D, Hamant O: **FibrilTool, an ImageJ plug-in to quantify fibrillar structures in raw microscopy images**. *Nat Protoc* 2014, **9**:457-463.

18. Pound MP, French AP, Wells DM, Bennett MJ, Pridmore TP: **CellSeT: novel software to extract and analyze structured networks of plant cells from confocal images**. *Plant Cell* 2012, **24**:1353-1361.

19. Smith RS, Guyomarc'h S, Mandel T, Reinhardt D, Kuhlemeier C, Prusinkiewicz P: **A plausible model of phyllotaxis**. *Proc Natl Acad Sci U S A* 2006, **103**:1301-1306.

20. Rolland-Lagan AG, Prusinkiewicz P: **Reviewing models of auxin canalization in the context of leaf vein pattern formation in *Arabidopsis***. *Plant J* 2005, **44**:854-865.

21. Grieneisen VA, Xu J, Maree AFM, Hogeweg P, Scheres B: **Auxin transport is sufficient to generate a maximum and gradient guiding root growth**. *Nature* 2007, **449**:1008-1013.

22. Band LR, Wells DM, Fozard JA, Ghetiu T, French AP, Pound MP, Wilson MH, Yu L, Li W, Hijazi HI *et al.*: **Systems analysis of auxin transport in the *Arabidopsis* root apex**. *Plant Cell* 2014, **26**:862-875.

23. Kenouche S, Perrier M, Bertin N, Larionova J, Ayadi A, Zanca M, Long J, Bezzi N, Stein PC, Guari Y *et al.*: **In vivo quantitative NMR imaging of fruit tissues during growth using spoiled gradient echo sequence**. *Magn Reson Imaging* 2014, **32**:1418-1427.

24. Verboven P, Herremans E, Helfen L, Ho QT, Abera M, Baumbach T, Wevers M, Nicolai BM: **Synchrotron X-ray computed laminography of the three-dimensional anatomy of tomato leaves**. *Plant J* 2015, **81**:169-182.

25. Dorca-Fornell C, Pajor R, Lehmeier C, Perez-Bueno M, Bauch M, Sloan J, Osborne C, Rolfe S, Sturrock C, Mooney S *et al.*: **Increased leaf mesophyll porosity following transient retinoblastoma-related protein silencing is revealed by microcomputed tomography imaging and leads to a system-level physiological response to the altered cell division pattern**. *Plant J* 2013, **76**:914-929.

26. Cloetens P, Mache R, Schlenker M, Lerbs-Mache S: **Quantitative phase tomography of *Arabidopsis* seeds reveals intercellular void network**. *Proc Natl Acad Sci U S A* 2006, **103**:14626-14630.

27. Lee K, Avondo J, Morrison H, Blot L, Stark M, Sharpe J, Bangham A, Coen E: **Visualizing plant development and gene expression in three dimensions using optical projection tomography**. *Plant Cell* 2006, **18**:2145-2156.

28. Flavel RJ, Guppy CN, Tighe M, Watt M, McNeill A, Young IM: **Non-destructive quantification of cereal roots in soil using high-resolution X-ray tomography**. *J Exp Bot* 2012, **63**:2503-2511.

29. Topp CN, Iyer-Pascuzzi AS, Anderson JT, Lee CR, Zurek PR, Symonova O, Zheng Y, Bucksch A, Mileyko Y, Galkovskyi T *et al.*: **3D phenotyping and quantitative trait locus mapping identify core regions of the rice genome controlling root architecture**. *Proc Natl Acad Sci U S A* 2013, **110**:E1695-E1704.

30. Mooney SJ, Pridmore TP, Helliwell J, Bennett MJ: **Developing X-ray computed tomography to non-invasively Image 3-D root systems architecture in soil**. *Plant Soil* 2012, **352**:1-22.

31. Moreno N, Bougourd S, Haseloff J, Feijo J: In *Imaging Plant Cells*. Edited by Pawley J. New York: Springer Science and Business Media; 2006.

32. Geldner N, Denervaud-Tendon V, Hyman DL, Mayer U, Stierhof YD, Chory J: **Rapid, combinatorial analysis of membrane compartments in intact plants with a multicolor marker set**. *Plant J* 2009, **59**:169-178.

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

In this paper the authors present the balloons segmentation of plant cells, as well as novel genetic constructs that can be used to 'seed' segmentation algorithms. Ratiometric analysis of nuclear reporters is also provided.

34. Truernit E, Bauby H, Dubreucq B, Grandjean O, Runions J, Barthelemy J, Palauqui JC: **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* 2008, **20**:1494-1503.

35. • Montenegro-Johnson TD, Stamm P, Strauss S, Topham AT, Tsagris M, Wood ATA, Smith RS, Bassel GW: **Digital single-cell analysis of plant organ development using 3DCellAtlas**. *Plant Cell* 2015, **27**:1018-1033.

A method to annotate 3D images in radially symmetric plant organs is presented. The method enables cell type recognition and geometric parameters to be extracted from confocal images based on a local coordinate system that follows the curvature of the organ.

36. Wuyts N, Palauqui JC, Conejero G, Verdeil JL, Granier C, Massonnet C: **High-contrast three-dimensional imaging of the *Arabidopsis* leaf enables the analysis of cell dimensions in the epidermis and mesophyll**. *Plant Methods* 2010:6.

37. Sena G, Frentz Z, Birnbaum KD, Leibler S: **Quantitation of cellular dynamics in growing *Arabidopsis* roots with light sheet microscopy**. *PLoS ONE* 2011:6.

38. Maizel A, von Wangenheim D, Federici F, Haseloff J, Stelzer EH: **High-resolution live imaging of plant growth in near physiological bright conditions using light sheet fluorescence microscopy**. *Plant J* 2011, **68**:377-385.

39. Furuta KM, Yadav SR, Lehesranta S, Belevich I, Miyashima S, Heo JO, Vaten A, Lindgren O, De Rybel B, Van Isterdael G *et al.*: ***Arabidopsis* NAC45/86 direct sieve element morphogenesis culminating in enucleation**. *Science* 2014, **345**:933-937.

40. Denk W, Horstmann H: **Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure**. *PLoS Biol* 2004, **2**:e329.

41. Shu X, Lev-Ram V, Deerinck TJ, Qi Y, Ramko EB, Davidson MW, Jin Y, Ellisman MH, Tsien RY: **A genetically encoded tag for correlated light and electron microscopy of intact cells, tissues, and organisms**. *PLoS Biol* 2011, **9**:e1001041.

42. • Dhondt S, Vanhaeren H, Van Loo D, Cnudde V, Inze D: **Plant structure visualization by high-resolution X-ray computed tomography**. *Trends Plant Sci* 2010, **15**:419-422.

The use of high resolution X-ray computed tomography provides cellular level imaging of an *Arabidopsis* hypocotyl in 3D.

43. Heisler MG, Ohno C, Das P, Sieber P, Reddy GV, Long JA, Meyerowitz EM: **Patterns of auxin transport and gene expression during primordium development revealed by live imaging of the *Arabidopsis* inflorescence meristem**. *Curr Biol* 2005, **15**:1899-1911.

44. Gooh K, Ueda M, Aruga K, Park J, Arata H, Higashiyama T, Kurihara D: **Live-cell imaging and optical manipulation of *Arabidopsis* early embryogenesis**. *Dev Cell* 2015, **34**:242-251.

45. • Liao CY, Smet W, Brunoud G, Yoshida S, Vernoux T, Weijers D: **Reporters for sensitive and quantitative measurement of auxin response**. *Nat Methods* 2015.

A more sensitive version of a transcriptional reporter of auxin response DR5v2 is presented, along with a ratiometric version of the DII-venus auxin reporter, R2D2.

46. Kawase T, Sugano SS, Shimada T, Hara-Nishimura I: **A direction-selective local-thresholding method, DSLT, in combination with a dye-based method for automated three-dimensional segmentation of cells and airspaces in developing leaves**. *Plant J* 2015, **81**:357-366.

47. Schiessl K, Kausika S, Southam P, Bush M, Sablowski R: **JAGGED controls growth anisotropy and coordination between cell size and cell cycle during plant organogenesis**. *Curr Biol* 2012, **22**:1739-1746.

48. Bassel GW: **Accuracy in quantitative 3D image analysis**. *Plant Cell* 2015, **27**:950-953.

49. Roeder AHK, Cunha A, Burl MC, Meyerowitz EM: **A computational image analysis glossary for biologists**. *Development* 2012, **139**:3071-3080.

50. Cunha A, Tarr PT, Roeder AHK, Altinok A, Mjolsness E, Meyerowitz EM: **Computational analysis of live cell images of the *Arabidopsis thaliana* plant**. *Comput Meth Cell Biol* 2012, **110**:285-323.

51. Sommer C, Straehle C, Koethe U, Hamprecht F: **ilastik: interactive learning and segmentation toolkit**. *IEEE International Symposium on Biomedical Imaging: From Nano to Macro*. 2011:230-233.

52. Lorensen WE, Cline HE: **Marching cubes: a high resolution 3D surface construction algorithm**. In *SIGGRAPH '87 Proceedings of the 14th Annual Conference on Computer Graphics and Interactive Techniques*. 1987:163-169.

53. Vernoux T, Brunoud G, Farcot E, Morin V, Van den Daele H, Legrand J, Oliva M, Das P, Larrieu A, Wells D *et al.*: **The auxin signalling network translates dynamic input into robust patterning at the shoot apex**. *Mol Syst Biol* 2011:7.

54. Runions A, Fuhrer M, Lane B, Federl P, Rolland-Lagan AG, Prusinkiewicz P: **Modeling and visualization of leaf venation patterns**. *ACM Trans Graph* 2005, **24**:702-711.

55. Jonsson H, Heisler MG, Shapiro BE, Meyerowitz EM, Mjolsness E: **An auxin-driven polarized transport model for phyllotaxis**. *Proc Natl Acad Sci U S A* 2006, **103**:1633-1638.

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

57. Vlad D, Kierzkowski D, Rast MI, Vuolo F, Dello Ioio R, Galinha C, Gan XC, Hajheidari M, Hay A, Smith RS *et al.*: **Leaf shape evolution through duplication, regulatory diversification, and loss of a homeobox gene**. *Science* 2014, **343**:780-783.

58. Kuchen EE, Fox S, de Reuille PB, Kennaway R, Bensmihen S, Avondo J, Calder GM, Southam P, Robinson S, Bangham A *et al.*: **Generation of leaf shape through early patterns of growth and tissue polarity**. *Science* 2012, **335**:1092-1096.

59. Rolland-Lagan AG, Remmler L, Girard-Bock C: **Quantifying shape changes and tissue deformation in leaf development**. *Plant Physiol* 2014, **165**:496-505.

60. Failmezger H, Jaegle B, Schrader A, Hulskamp M, Tresch A: **Semi-automated 3D leaf reconstruction and analysis of trichome patterning from light microscopic images**. *PLoS Comput Biol* 2013, **9**:e1003029.

61. Scarpella E, Marcos D, Friml J, Berleth T: **Control of leaf vascular patterning by polar auxin transport**. *Genes Dev* 2006, **20**:1015-1027.

62. Dumais J, Kwiatkowska D: **Analysis of surface growth in shoot apices**. *Plant J* 2002, **31**:229-241.

63. Williams MH, Green PB: **Sequential scanning electron-microscopy of a growing-plant meristem**. *Protoplasma* 1988, **147**:77-79.

64. •• Uyttewaal M, Burian A, Alim K, Landrein BT, Borowska-Wykret D, Dedieu A, Peaucelle A, Ludynia M, Traas J, Boudaoud A *et al.*: **Mechanical stress acts via katanin to amplify differences in growth rate between adjacent cells in *Arabidopsis***. *Cell* 2012, **149**:439-451.

The sequential replica method is used to extract cell geometry on the 2.5D meristem surface. The method is combined with confocal imaging to simultaneously track gene expression. The impact of cell to cell mechanical stresses mediated by katanin is integrated into a computational model of meristem development.

65. • Shapiro BE, Tobin C, Mjolsness E, Meyerowitz EM: **Analysis of cell division patterns in the *Arabidopsis* shoot apical meristem**. *Proc Natl Acad Sci U S A* 2015, **112**:4815-4820.

2.5D surface projections are used to analyze cell division patterns in the growing shoot meristem.

66. Hejnowicz Z, Karczewski J: **Modeling of meristematic growth of root apices in a natural coordinate system**. *Am J Bot* 1993, **80**:309-315.

67. Cruz-Ramirez A, Diaz-Trivino S, Blilou I, Grieneisen VA, Sozzani R, Zamioudis C, Miskolczi P, Nieuwland J, Benjamins R, Dhonukshe P *et al.*: **A bistable circuit involving SCARECROW-RETINOBLASTOMA integrates cues to inform asymmetric stem cell division**. *Cell* 2012, **150**:1002-1015.

68. • De Rybel B, Adibi M, Breda AS, Wendrich JR, Smit ME, Novak O, Yamaguchi N, Yoshida S, Van Isterdael G, Palovaara J *et al.*: **Plant development. Integration of growth and patterning during vascular tissue formation in *Arabidopsis***. *Science* 2014, **345**:1255215.

This work uses 3D images of developing embryos to examine the intracellular connectivity of the early *Arabidopsis* embryo, and how this influences genetic processes underlying patterning.

69. Nakayama N, Smith RS, Mandel T, Robinson S, Kimura S, Boudaoud A, Kuhlemeier C: **Mechanical regulation of auxin-mediated growth**. *Curr Biol* 2012, **22**:1468-1476.

70. Swarup R, Kramer EM, Perry P, Knox K, Leyser HMO, Haseloff J, Beemster GTS, Bhalerao R, Bennett MJ: **Root gravitropism requires lateral root cap and epidermal cells for transport and response to a mobile auxin signal**. *Nat Cell Biol* 2005, **7**:1057-1065.

71. Hamant O, Heisler MG, Jonsson H, Krupinski P, Uyttewaal M, Bokov P, Corson F, Sahlin P, Boudaoud A, Meyerowitz EM *et al.*: **Developmental patterning by mechanical signals in *Arabidopsis***. *Science* 2008, **322**:1650-1655.

72. Dupuy L, Mackenzie J, Rudge T, Haseloff J: **A system for modelling cell-cell interactions during plant morphogenesis**. *Ann Bot* 2008, **101**:1255-1265.

73. Boudon F, Chopard J, Ali O, Gilles B, Hamant O, Boudaoud A, Traas J, Godin C: **A computational framework for 3D mechanical modeling of plant morphogenesis with cellular resolution**. *PLoS Comput Biol* 2015:11.

74. • Cerutti G, Godin C: **Meshing meristems — an iterative mesh optimization method for modeling plant tissue at cell resolution**. *BIOIMAGING*. 2015.

A tool to create quality meshes from 3D segmentation of plant samples is presented. Its use is demonstrated on a segmentation of a shoot apical meristem.

75. Peret B, Middleton AM, French AP, Larrieu A, Bishopp A, Njo M, Wells DM, Porco S, Mellor N, Band LR *et al.*: **Sequential induction of auxin efflux and influx carriers regulates lateral root emergence**. *Mol Syst Biol* 2013:9.

76. Yadav RK, Perales M, Gruel J, Ohno C, Heisler M, Girke T, Jonsson H, Reddy GV: **Plant stem cell maintenance involves direct transcriptional repression of differentiation program**. *Mol Syst Biol* 2013, **9**:654.

77. Chakraborty A, Perales MM, Reddy GV, Roy-Chowdhury AK: **Adaptive geometric tessellation for 3D reconstruction of anisotropically developing cells in multilayer tissues from sparse volumetric microscopy images**. *PLOS ONE* 2013:8.