INVITED REVIEW

PLANT MORPHOLOGY vol. 33 pp. 15-23

Multidisciplinary bioimaging approach to study plant morphogenesis

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Summary: Morphology of plants is controlled by coordinated patterns of cell division and growth. Understanding the molecular mechanisms that regulate plant architecture requires careful observation and detailed analysis at the level of cells and tissues. In this review, we introduce recently developed microscopy protocols and computational tools to obtain and analyze cellular level data for research on plant morphology and development. We mainly focus on protocols for imaging plant structures by confocal laser scanning microscopes, followed by quantitative analysis based on 3D segmentation of cells layers/tissues, and computational modelling to explore fundamental questions in plant developmental biology.

Key words: 3D segmentation, cell geometry, morphogenesis, MorphoGraphX, quantitative bioimage analysis

INTRODUCTION

Multicellular organisms are composed of cells forming patterns. To understand how these patterns are generated and maintained, it is necessary to observe and quantify the arrangement and geometry of cells, organelles, gene expression patterns and protein localization at cellular and subcellular level. State of the art imaging methods enable detailed observation of plant organs and structures in 3-dimensions (3D) or in 4D (over time in 3D). The analysis of such images allows the extraction of detailed morphological information. Based on a quantitative analysis, mathematical and computational models can be developed that aim to provide an integrated understanding of cellular-level behaviors that produce the morphology of the multicellular body. In recent years, great advances in the abilities of microscopy and image analysis have made it possible to observe spatial-temporal dynamics during morphogenesis at cellular and subcellular level. Imaging data obtained in this manner, and its quantitative analysis combined with computer modeling is termed "Computational Morphodynamics", which has gathered increasing interest in the field of plant developmental biology (Chickarmane et al. 2020). In this review, we introduce recent advances in such multidisciplinary approaches in plant developmental biology, especially focusing on state-of-the-art microscopy techniques and the analysis of resulting images via 3D segmentation of cells using the MorphoGraphX software platform as an example of a widely used software within the plant research community (Barbier de Reuille et al. 2015).

MULTI-DIMENSIONAL IMAGE ACQUISITION BY LASER SCANNING MICROSCOPES

Confocal laser scanning microscopes are commonly used for the observation of fluorescently marked cells, subcellular organelles, and gene expression and protein localization patterns. Unlike physical sectioning of the specimen, optical sectioning with a confocal laser scanning microscope enables the user to acquire high resolution images that can be reconstructed in 3D without destroying the samples. Various types of laser microscopes are used for bioimaging. Point scanning and spinning disc microscopes are the most commonly used, whereas light sheet, multiphoton and super resolution microscopes are increasingly used in recent years. The latter systems may achieve improved optical sectioning without the use of a pinhole at a conjugate focal plane (Gooh et al. 2015, von Wangenheim et al. 2017, Ovečka et al. 2018, Haas et al. 2020) .

There are two main types of image acquisition: fixed sample imaging (snapshot imaging) and *in vivo* imaging. The choice between the two comes with corresponding pros and cons, with technical limitations often playing a decisive role in which method is selected. Plant cells are composed of various compounds that may display auto-fluorescence, e.g. chlorophyll (Donaldson 2020), and show different refractive indices, resulting in disturbed marker signal detection. Fixation followed by treatment with a clearing solution with a refractive index matching the sample can potentially solve such problems. This technique enables the observation of the morphology of cells located deep inside of organs. For example, modified pseudo-Schiff propidium iodide (mPS-PI) staining is a protocol combining PI staining of the cell wall followed by clearing by chloral hydrate (Truernit et al. 2008). In addition to various "naked/exposed" organs, which are relatively easy to access and observe such as leaves and roots, it enables the imaging of 3D cellular morphology of, for instance, plant embryos developing inside ovules (Truernit et al. 2008, Yoshida et al. 2014). While mPS-PI staining can be combined with the observation of GUS (beta-glucuronidase) reporter gene expression, additional fluorescent marker proteins are not preserved by this protocol. Another clearing protocol, ClearSee, can be applied for multicolor imaging of various fluorescent proteins and cellular components stained by dyes (Kurihara et al. 2015, Ursache et al. 2018, Tofanelli et al. 2019).

While a combination of fixation and clearing allows the acquisition of high resolution static 3D images, observation of temporal morphological changes requires repeated imaging of live specimens. Generally, somatic plant cells are surrounded by interconnected rigid cell walls and therefore are largely immobile with respect to their direct neighbors. As a result, cell growth and proliferation are major determinants of development, which can

be only quantitatively approached through time-lapse imaging of live specimens. A common protocol for visualization of the outlines of cells is to use dyes to stain the cell wall (e.g. Propidium iodide, Calcofluor white, Renaissance 2200) or plasma membrane (e.g. FM4-64). Another conventional method is the introduction of transgenes expressing fluorescent membrane marker proteins (Kierzkowski et al. 2012, De Rybel et al. 2013, 2014, Ursache et al. 2018, Kierzkowski et al. 2019, Tofanelli et al. 2019, Wolny et al. 2020). Together with cell outlines, also expression patterns of genes and proteins can be observed and later processed to quantify the cellular signals.

For *in vivo* and time-lapse 3D imaging in particular, it is important to grow samples in an environment as close as possible to the natural conditions. Repeated imaging typically generates substantial stress in form of phototoxicity in samples, thus it is essential to minimize other sources of stress. Therefore, the control of environmental light, temperature, nutrition, gas exchange, as well as sample orientation with respect to incident light and gravity need to be considered carefully. von Wangenheim et al. (2017) established a live imaging system using a confocal laser scanning microscope with vertically growing roots. This system made it possible to grow seedlings vertically, i.e. their natural orientation with respect to earth gravity. This was combined with TipTracker, a program to automatically track the root tip, which largely solves the issue of root tip displacement by growth during time-lapse observation. A LED illumination system is attached to the microscope stage, which allows directional lighting of the samples. Using this time-lapse imaging system, it is possible to image roots for quantitative analysis as exemplified by its use in studying restoration of cellular patterns after wounding (Hoermayer et al. 2020).

Light sheet fluorescence microscopy is another type of microscopy, that is useful for live imaging of animal and plant bodies (Ovečka et al. 2018, Valuchova et al. 2019). Specimens are illuminated by a planar sheet of light which generates fluorescence over an entire optical section, which in contrast to point scanning laser systems enables multidimensional imaging at high speed and with minimal photo damage. In plants, it has been used for time-lapse imaging of roots which are relatively transparent organs due to absence of chloroplasts (Ovečka et al. 2018).

Two-photon excitation microscopy is used for deep imaging of fixed and live specimens. Samples are excited by less scattering near-infrared light only at the focal plane of the observation, which reduces photo-bleaching, photo-toxicity and autofluorescence (Gooh et al. 2015). Therefore, this methodology can be useful for imaging of delicate samples at great depth. Gooh et al. (2015) established a system to perform time-lapse observation of *Arabidopsis* embryos from zygote to heart stage with the aforementioned imaging system, combining two-photon microscope and a microfluidic device. The use of two-photon microscopy enables imaging the live *Arabidopsis* embryo intact inside the ovule while this is not possible with any of the previously mentioned microscopes. The microfluidic device was used to physically fix ovules, preventing the movement of the samples between imaging sessions, which is a commonly encountered problem in time-lapse imaging, while it also administers nutrients and oxygen to maintain development (Gooh et al. 2015).

2.5D AND 3D SEGMENTATION

Segmenting the boundaries of cells in tissues/organs is a necessary step in determining cell size and geometry for quantitative cellular-level studies. Besides that, a cellular segmentation also allows a further analysis of the observation and quantification of gene expression and protein localization patterns, subcellular organelles and the arrangement of cells in organs. Many computational tools for segmentation and image processing have been developed (see https://www.quantitative-plant.org/software for a comprehensive overview, Fernandez et al. 2010, Schindelin et al. 2012, Schneider et al. 2012, Schmidt et al. 2014, Barbier de Reuille et al. 2015, Legland et al. 2016, Martinez et al. 2018, Berg et al. 2019, Selka et al. 2017). In recent years, one of the popular tools among the plant biology community has been MorphoGraphX (MGX, https://www.morphographx.org/), an open-source, customizable software platform for quantitative analysis of 3D image datasets for fixed and time-lapse images (Barbier de Reuille et al. 2015). MGX was originally developed to perform cellular segmentations on curved organ surfaces (so-called 2.5D segmentations, i.e. a non-flat 2D surface) and subsequent quantitative analyses.

Using 3D images, MGX can extract curved surfaces of cellular structures (Figure 1A, B). The 3D data just below this surface, such as outlines of cells and fluorescent signals is then projected on this surface and used for segmentation (Figure 1C). The 2.5D data can be used for the quantification of cellular geometry as well as cell lineage tracing and to observe cellular morphological changes, cell divisions, and gene/protein expression patterns over multiple time points (Figure 1D, Barbier de Reuille et al. 2015). For instance, 2.5D segmentations have been used to study cellular morphological changes in early leaf development (Vlad et al. 2014, Kierzkowski et al. 2019, Zhang et al. 2020), sepals (Hervieux et al. 2015, Zhu et al. 2020) and shoot apical or floral meristems (Kierzkowski et al. 2012, Louveaux et al. 2016, Kinoshita et al. 2020). In many cases 2.5D segmentation is preferred over the much more time consuming and more error prone 3D segmentation. In addition, in practice often the 2.5D segmentation provides a sufficiently accurate representation of the cells and organs, especially on the surface of curved organs where the traditional method of a 2D projection would introduce distortions and inaccuracies (Kierzkowski et al. 2012, Vlad et al. 2014, Louveaux et al. 2016, Barbier de Reuille et al. 2015, Hervieux et al. 2015, Hong et al. 2016, Kierzkowski et al. 2019). Moreover, in many plant developmental studies the overall organ growth rates can be reasonably estimated by quantification of organ surface growth. For these reasons the 2.5D segmentation can be easier and therefore is more commonly used for quantitative analysis (Kierzkowski et al. 2012, Vlad et al. 2014, Hervieux et al. 2015, Hong et al. 2016, Louveaux et al. 2016, Kierzkowski et al. 2019, Sapala et al. 2018).

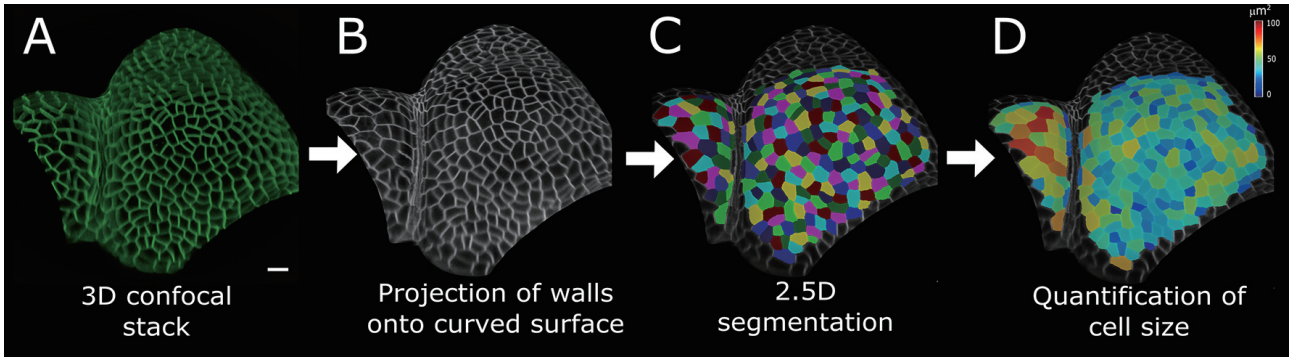


Figure 1 The workflow of 2.5D segmentation. (A) 3D confocal stack of an Arabidopsis inflorescence shoot apical meristem as visualized by the 3D renderer of MGX. Cell walls were stained by propidium iodide (green). (B) After the extraction of the curved surface of the 3D stack, the cell wall signal below the surface is projected on it (white). (C) Next, cells are segmented on this curved image by the watershed algorithm. Cells are colored randomly. (D) Heatmap of cell size based on 2.5D segmentation. Scale bar 10 μm.

Besides the 2.5D segmentation, MGX also allows the generation and analysis of 3D segmentations (Figure 2A, Bassel et al. 2014, Yoshida et al. 2014, Vijayan et al. 2020). A 3D segmentation provides a complete description of cellular morphology and therefore is the most detailed information for quantitative bioimage analysis. In contrast, 2D observations of a volumetric organ must be correctly assessed, otherwise the results can be misleading. For example, during *Arabidopsis* embryo development, the embryo forms a second cell layer with coordinated cell divisions when developing into the 16cell stage. Based on the observation of 2D histological sections, it was previously believed that cells at the inner layer are bigger than cells at the outer layer. However, analysis of 3D segmented cells showed that the outer cells are bigger than the inner cells (Yoshida et al. 2014). 3D segmentation requires higher quality microscopy images because the outlines of cells have to be clear in all directions. In particular obtaining sufficient image quality in the direction along the optical axis is often challenging (the 'z' direction along which images are stacked). Increasing the scanning resolution is not always helpful as it leads to reduced signal per voxel that needs to be compensated through an increase in imaging time that may result in bleaching and sample damages.

In MGX, the 3D segmentation process is semi-automatic; it consists of a combination of an automatic segmentation step by the MGX software (which uses the watershed algorithm of the ITK library, Yoo et al. 2002) followed by a manual step to correct segmentation errors. The amount of correction needed correlates with the quality of the confocal images and the number of cells in the sample. Image quality here corresponds to how well the captured signal represents the actual cell wall or membrane. Images of lower quality often show higher level of noise which results in less clear cell walls, as well as cell outlines with gaps or holes. Therefore, it is important to optimize the imaging procedure carefully before moving to the image acquisition step. The optimization is mainly needed for followings issues: 1) protocols for sample preparation (e.g. live or fixed), 2) types of microscopes used (e.g. single-photon or two-photon microscope system), 3) finding the optimal microscope settings. Additional image processing after microscopy, such as deconvolution may be required to improve the quality of images (Sarder and Nehorai, 2006). Afterwards the images are ready to be loaded into MGX. In the segmentation process, the software fills all cells with color labels (Figure 2A). From this labeled 3D image MGX generates triangulated meshes, which are required for the further data analysis and the final form of the 3D data, which subsequently can be used for quantitative analysis. For examples of different 3D segmented organs, see Figure 2I-J.

The 3D segmented data can display detailed morphology of outer and inner cells from all directions (Figure 2B, C). While the labels and meshes are colored randomly during the automatic segmentation process (Figure 2A, B, C), 3D cell meshes can be annotated and colored based on the cell types and/or cell lineages (Figure 2A, the rightmost image). Annotation of 3D segmented cells based on their identity and position in organs is required to establish their context and to extract their biological features for further analysis (Figure 2A, the rightmost image).

For samples with large numbers of cells, it is not practical to manually annotate cells. Automatic or semi-automatic annotation pipelines of 3D segmented tissues/organs for making 3D single cell atlases are reported for various plant organs and structures; in shoot apical meristem (Montenegro-Johnson et al. 2019), root (Schmidt et al. 2014, Montenegro-Johnson et al. 2015), leaf (Selka et al. 2017), ovule (Vijayan et al. 2020) and mature embryo (Montenegro-Johnson et al. 2015). These annotation pipelines are useful for the analysis of individual plants but also for the analysis of large numbers of samples such as plant populations.

A multitude of different quantification functionalities has been implemented in MGX. Apart from cell morphology, it is also possible to quantify the orientation of microtubules by projecting the signals of microtubules on to the labeled mesh (Sampathkumar et al. 2014, Hofhuis et al. 2016) as well as to segment and quantify the volume of organelles, such as vacuoles (Scheuring et al. 2016).

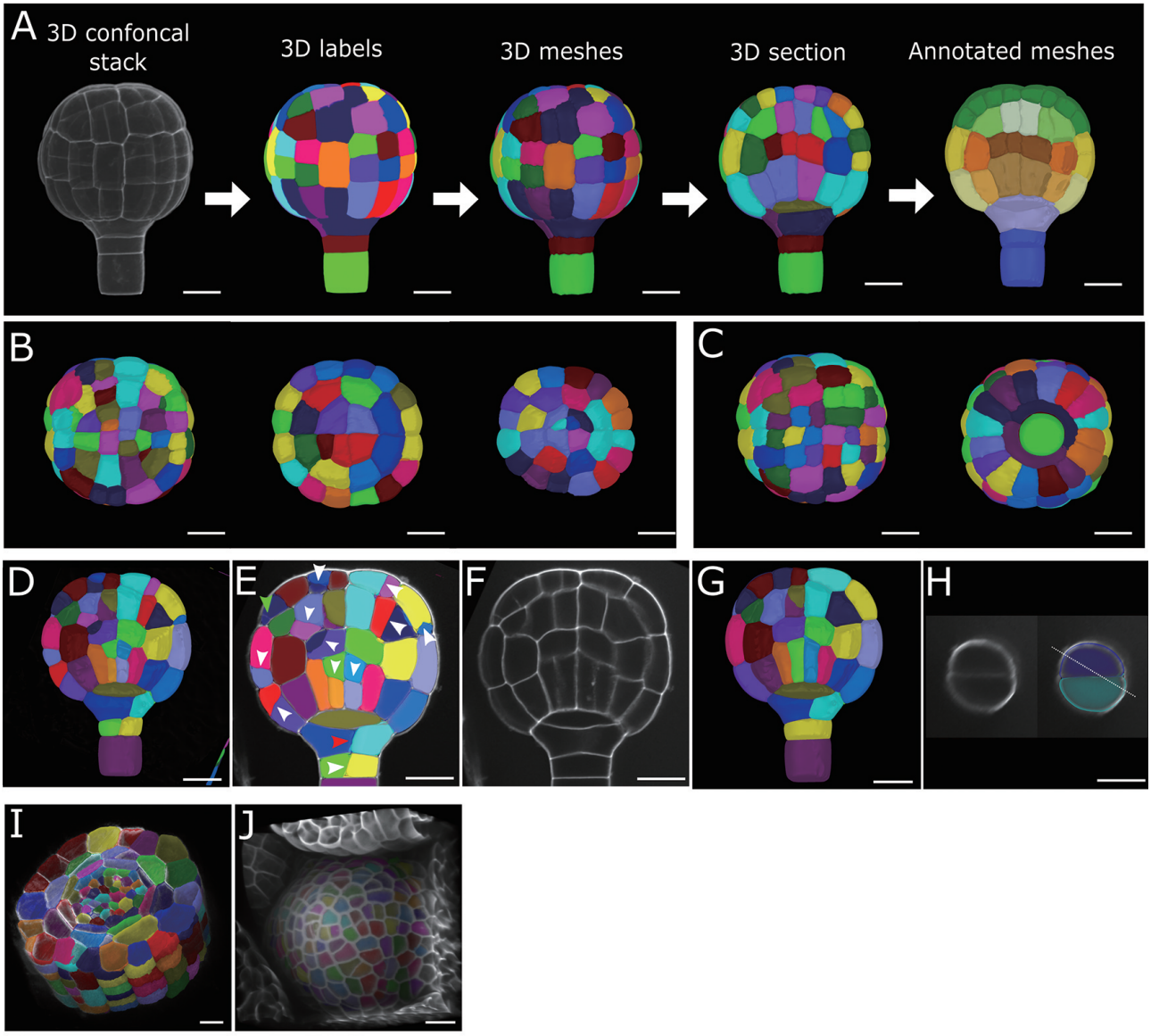


Figure 2 3D segmentation of cells by MorphoGraphX. (A) The workflow for generating 3D segmented data of the Arabidopsis embryo at the globular stage. The first step of segmentation typically involves blurring the 3D confocal image containing the cell wall signal (not shown; the extent of which can vary depending on the images) to reduce the noise. Then follows the automatic seeding and the segmentation by the watershed algorithm, which fills each cell with unique labels colored for visualization (2nd image from the left). For the further analysis, 3D meshes are then created from the segmented stack (3rd image from the left). A longitudinal section of 3D segmented embryo (4th image from the left). Cells can be colored based on cell types (the rightmost image). Full 3D segmented meshes can be observed from every direction; transverse sections from apical to basal directions (from left to right shown in B) and from top and bottom view (C). (D) Examples of mis-segmented cells; meshes are shown. (E) Overlay of labels and (F) original confocal stack. Note that white arrows show mis-segmented cell walls. Cell walls indicated by red and green arrows are correctly segmented. (G) Correctly segmented embryo. (H) Transverse view of overlay of confocal stack and meshes. The cell wall dividing the columella cell is visible in the transverse section (H) but difficult to find in longitudinal section (F). In H, the dotted line shows the position of the section shown in F. (I) 3D segmented transverse section of root and (J) shoot apical meristem of Arabidopsis. The confocal stack and meshes are overlayed. Cell outlines in the confocal images are shown in white. Scale bars 10 μm.

VERIFYING AND IMPROVING THE 3D SEGMENTATION QUALITY

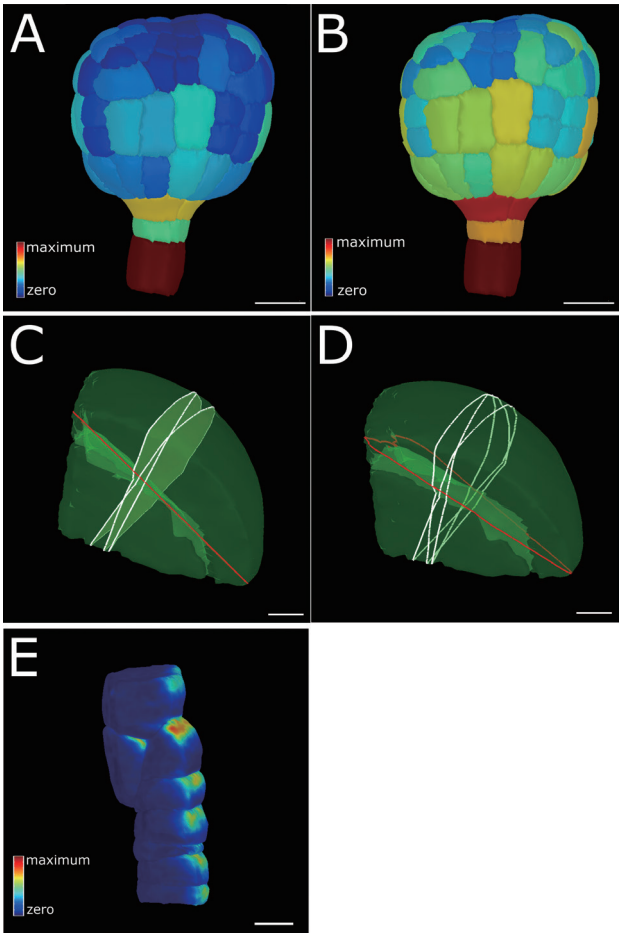
3D segmentation can be a powerful tool to extract information from imaging data. For that reason, the reliability of 3D segmentation is important. In full 3D segmentation, errors are often difficult to recognize as cells need to be evaluated from different angles (Figure 2D, E, F, G) in contrast to 2D or 2.5D segmentation, which is much simpler (Figure 1). Figure 2D shows examples of such mis-segmentation; although the arrangement of cells seems not unnatural, it contains in a total of 11 errors. Sometimes, the cell wall signal of the neighboring cells appears as a newly formed division wall, while such a wall is absent at the focal plane (Figure 2E, green arrow). Also, the cell wall (or plasma membrane) signal can be difficult to detect in a given direction but more visible in other directions. For example, in Figure 2E and 2F, the cell wall dividing columella cell is hardly visible, however, the transverse section shows that the cell is actually composed of two columella cells (Figure 2H). To avoid segmentation

mistakes, the 3D segmented data must be checked from multiple angles thoroughly, which is the most time-consuming part of the 3D segmentation. The weak signal of the cell wall or plasma membrane causes the outline of segmented cells to be mis-shaped, another source of distortion that has to be considered carefully. However, at present there are no alternatives to a supervised semi-automated 3D segmentation with manual corrections made during or after processing. In our experience, especially for the samples containing around 100 cells the manual correction step is already substantial. Ideally every 3D segmented cells should be checked to detect all possible mis-segmentations. This is particularly important for experiments that aim to detect and quantify cell divisions as newly formed division walls can have faint signals that can go undetected and also has the effect that artefacts in the image are more likely to be mistakenly detected as division walls. However, in practice, it is difficult to check the segmentation of every cell when the samples contain hundreds or thousands of cells. Therefore, depending on the purpose of the experiment, it is also possible to average out the noise introduced via mis-segmentations using multiple samples and a large number of cells. In any case it is advisable to manually inspect at least some of the samples to get an idea of the magnitude of error in quantification that is introduced due to the mis-segmentations.

Several potential methods exist to increase the accuracy of 3D segmentation and to make the segmentation process faster and easier. The z-axis of confocal image stacks suffers from less resolution compared to the lateral x-y axes. This situation can be improved by combining 3D stacks taken from multiple angles, which has been shown to significantly improve 3D segmentation (Fernandez et al. 2010, Ovečka et al. 2018, Valuchova et al. 2019). Recently several powerful tools for improved automated cell segmentation were published. For instance, the machine-learning platform ilastik, contains multiple workflows for image segmentation and other image processing algorithms (Berg et al. 2019). Another tool called PlantSeg is a deep-learning based pipeline for 2D and 3D segmentation of cells that may approach human performance (Wolny et al. 2020). The software uses a convolutional neural network which, after sufficient training, is able to predict the outlines of cells to a high level of accuracy. This improved cell wall signal can subsequently be used for segmentation (Wolny et al. 2020). The obtained data from these tools can be directly plugged into other image analysis software (MGX, Fiji etc,) for data analysis.

QUANTITATIVE DATA ANALYSIS AND MODELLING

Once the 3D cellular segmentation is generated, it can be used to quantify a variety of cell characteristics and features. Quantitative analysis provides morphological measurements during normal development that can be used for high-resolution phenotyping of mutants. Various morphological features can be quantified, among them are cell volume, cell surface area, cell division wall analysis and cell shape anisotropy (Figure 3A, B, C, D) (Montenegro-Johnson et al. 2015, 2019, Hernandez-Lagana et al. 2020, Vijayan et al. 2020). Furthermore quantification of gene expression in cellular and subcellular level and simple visualization of protein localization at cellular resolution can be performed on a 3D segmented cellular mesh (Figure 3E, Montenegro-Johnson et al. 2015, Chakrabortty et al. 2018, Yoshida et al. 2019, Vijayan et al. 2020).



Figue3 Examples of analysis of 3D segmented data. Analyses of (A) cell volume, (B) cell surface area, (C, D) division wall on a 3D meshes and (E) visualization of corner localized protein by MorphoGraphX In A and B, cell volumes or cell surface areas are shown by a heatmap. In C and D, planes marked by red outlines show a planar approximation of the real division plane, and the planes marked by white outlines are the smallest walls dividing the mother cell. In E, the 3D localization of a corner localized protein, SOSEKI1 in the root epidermal cells of RPS5A-SOK1-YFP plants (Yoshida et al. 2019) is shown by heatmap. Scale bars are 10 μm (A, B, E) or 2 μm (C, D).

While such descriptive knowledge is important to develop a detailed timeline of morphogenesis, an optimal analysis of such data should contribute to a mechanistic and integrated view of the developmental process under study. Such an effort is often aided by computational modelling. To understand morphogenesis of a multicellular body, it is required to analyze the spatial and temporal interactions between cellular patterns and gene/protein expression. This mutual interaction can take unintuitive and highly complex forms that can only be approached via computational modeling. While there are many quantitative analysis and modeling-based studies that have been performed using 2.5D segmentation (Kierzkowski et al. 2012, Hervieux et al. 2015, Sapala et al. 2018, Kierzkowski et al. 2019), there are

far fewer studies based on full 3D segmented data. We will briefly review a study where the combination of detailed 3D imaging, quantitative analysis and modelling led to a more integrated view of the process of cell division in plants.

Orientation of the cell division plane is an important determinant for plant morphogenesis. Application of 3D imaging and computational modelling to the study of cell division orientation is a good example of the capabilities and impact of the computational morphodynamics in studying cellular-level processes. The shortest wall rule is a classical hypothetical rule explaining orientations of cell divisions; it states that plant cells divide like soap bubbles, by finding the shortest division plane that goes through the center of a cell (Errera 1888, Yoshida et al. 2014). Based on the quantitative data obtained from 3D segmented embryonic cells, a computational model demonstrated that while shortest wall accounts for some embryonic divisions, cell divisions deviate from this rule by responding to auxin signaling, which leads to asymmetric cell division. This indicated that a simple default rule likely couples division orientation to cell geometry, and that genetic regulation create cellular patterns by overriding the default division rule (Yoshida et al. 2014). Quantification of microtubules combined with computational modeling that simulate self-organization of cortical microtubule arrays based on 3D segmented embryonic cells, has shown that the cell division plane orientation at early embryogenesis is predictable by considering three factors; embryonic cell geometry, effect of cell edges on microtubule catastrophes (microtubule shortening) and developmental cues such as auxin mediated microtubules stability (Chakrabortty et al. 2018). Cell division orientation was also examined in other plant species as well as animal cells; analysis of maize leaf epidermal layers and *Caenorhabditis elegans* embryos by different computational tools for 3D segmentation, indicated that the shortest wall rule is applicable in these species also (Martinez et al. 2018). Further analysis of different biological systems will reveal to what extent this rule can explain the various types of cell division observed in plants.

Another successful example is the study of the effect of cell geometry on morphogenesis during seed germination (Bassel et al. 2014). In the tip of the radicle when it emerges during germination, it has been observed that the center of cell expansion is spatially displaced from the expression domain of growth promoting genes. Using a 3D mechanical model of pressurized cells, it was shown that by taking into account the influence of cell geometry, the aforementioned displacement can be accounted for. Thus, the use of computational modeling led to insights and mechanistic understanding of a complex biological phenomenon.

ISSUES FOR THE FUTURE

Taken together, bioimaging tools and multidisciplinary approaches described in this review are effective when applied to provide answers to developmental questions in plant science. Currently there are several outstanding issues. Efforts to address these issues can greatly improve the feasibility and outcome of such methods. While many bioimaging and image analysis protocols have been developed and published in recent years, these software tools often perform similar main functionalities such as image processing steps, and quantification of basic cell properties. However, it is often unclear how to systematically compare the reliability, performance and sustainability of these software (Lobet 2017). Therefore, researchers often have to find out for themselves the computational tools and software best suited for their work. On top of that, it often remains unclear to what extent these tools can be independently and correctly used without the direct support of the original software developers, since it can be challenging and time consuming for an experimental biologist to install and configure these tools. Their interfaces are not always user-friendly and often difficult to master. In addition, the data analysis demanded by the user generally is a complex multi-step process rarely well documented in a software manual. These complications can lead to incorrect usage of software and/or incorrect interpretation of results. As such close communication and collaboration with the original developers and the user community is essential to prevent such problems. While for some software the developer and user communities are active and can be easily approached for help and advice, this is not the case for most.

Another unsolved problem concerns the reliability of the 3D segmentation data. There is currently no system to consistently and objectively evaluate the quality of such segmentations and their quality can only be assessed by personal experience of individual researchers. Image processing software can generate beautiful looking digital views of 3D segmented cells. But to what extent do these reflect the reality? As shown in Figure 2D-H, mistakes of 3D segmentation made during data processing are not easily visible. After having created the segmentation, often the original confocal stacks are no longer visualized, and it can be very challenging for other researchers to check the segmentation against original images. A similar problem can be caused by other image processing pipelines such as automatic cell annotation tools. While such issues do not necessarily have a large effect on the outcome of the analysis, they are additional sources of noise in the data. For this reason, it remains important to perform an initial manual assessment of the segmentation and annotation errors and their impact. In the coming years, it would be useful to promote more discussions about the quality of 3D data within the scientific community, so that such data can be easily verified using a common procedure.

In summary computational tools for processing and analyzing cellular and subcellular level images are powerful and attractive and have enabled a much greater understanding of morphogenesis. However, their application requires highly optimized experimental procedures, high quality images and the informed use of the often complex software. Finally, the researcher as a user of such data analysis platforms has to be aware of their limitations to draw the correct conclusions from their results.

ACKNOWLEDGEMENT

This study was supported by a Marie Curie Fellowship (contract 753138) to S.Y. and a Max Planck core grant. We thank Dr. Richard Smith (John Innes Centre), Prof. Miltos Tsiantis, Dr.

Peter Huijser, Dr. Ton Timmers (Max Planck Institute for Plant Breeding Research) for comments on the manuscript.

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Received: 2 February 2021/ Accepted: 8 April 2021