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INVITED REVIEW

Quantitative analysis of 3D cellular geometry and modelling of the Arabidopsis embryo

Saiko Yoshida[1] | **Dolf Weijers**[2]

[1]Department of Comparative Development and Genetics, Max Planck Institute for Plant Breeding Research, Köln, Germany

[2]Laboratory of Biochemistry, Wageningen University, Wageningen, the Netherlands

Correspondence
S. Yoshida, Department of Comparative Development and Genetics, Max Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, Köln D-50829, Germany.
Email: yoshida@mpipz.mpg.de

D. Weijers, Laboratory of Biochemistry, Wageningen University, Stippeneng 4, 6708 WE Wageningen, the Netherlands.
Email: dolf.weijers@wur.nl

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Abstract

As many multicellular organisms, land plants start their life as a single cell, which forms an embryo. Embryo morphology is relatively simple, yet comprises basic tissues and organs, as well as stem cells that sustain post-embryonic development. Being condensed in both time and space, early plant embryogenesis offers an excellent window to study general principles of plant development. However, it has been technically challenging to obtain high spatial microscopic resolution, or to perform live imaging, that would enable an in-depth investigation. Recent advances in sample preparation and microscopy now allow studying the detailed cellular morphology of plant embryos in 3D. When coupled to quantitative image analysis and computational modelling, this allows resolving the temporal and spatial interactions between cellular patterning and genetic networks. In this review, we discuss examples of interdisciplinary studies that showcase the potential of the early plant embryo for revealing principles underlying plant development.

KEYWORDS
computational modelling, quantitative image analysis, imaging, plant embryogenesis

1 | INTRODUCTION

A fundamental question in developmental biology is how the shapes of multicellular organisms are formed through the controlled growth and division of individual cells, and how these processes are coordinated. The cell is the smallest unit of life. A group of cells with the same function forms a tissue, and a group of different tissues composes an organ, which contributes to the body of a multicellular organism. Geometry and coordinated arrangements (patterns) of cells determine the shape of a multicellular body. Plant cells are surrounded by cell walls that restrict their mobility. Therefore, the rate and orientation of cell division and cellular growth are major factors in controlling the shape of the plant body. For this reason, it is of central importance in our understanding of plant development to visualise and analyse cellular morphology. In the past decade, advances in microscopy and bioimaging tools have enabled the visualisation of cell geometry and gene/protein expression patterns in a multidimensional manner – 3 spatial dimensions (3D) and time (4D) – to perform quantitative analysis. In addition to improved strategies for high-resolution imaging, novel approaches in computational modelling based on quantitative data

can solve spatial and temporal interplays of cellular patterning and gene/protein expression. In this review, we focus on some examples showcasing recent advances in microscopy-based imaging and computational data analysis as applied to studying the earliest phase of plant life – embryogenesis.

1.1 | The plant embryo as a model of morphogenesis

Multicellular organisms generally start their life as a single cell. The process of increasing organismal complexity as the organism grows is among the most fundamental ones in biology: the origin of complex life. In many species, this process is encapsulated in embryogenesis following fertilisation: reiteration of the life cycle from a single cell. Arguably, most of the developmental events that mark the life of an animal or plant are condensed into a brief window of time, and therefore, embryogenesis offers a good window into the fundamentals of organismal development. Here, we discuss plant embryogenesis, where the end product (in seed plants) is a mature embryo that has the major organs and tissues, as well as stem cells to sustain later development. We discuss the process through the lens of the model flowering plant *Arabidopsis thaliana*, which has been subject of intensive genetic, molecular and microscopic observation. In brief, the Arabidopsis zygote grows along an apical–basal axis and divides to generate a small apical cell that generates most of the embryo, and a basal cell that will generate a file of cells that essentially connects the embryo to the seed (Figure 1A). The apical cell divides a number of times until the 8-cell stage, after which distinct outer and inner cell layers are formed along the central-peripheral axis (Figure 1A). Approximately simultaneously, the embryo becomes partitioned into apical and basal domains, that each forecast the later shoot and root domains of the mature embryo and seedling. At early globular stage, inner basal embryonic cells are specified into the cell types and stem cells of the root meristem, vasculature and ground tissue stem cells. During transition and early heart stage, the shape of the embryo changes from spherical to heart, while cotyledons and the shoot meristem are established, along with the internal cell types and domains (e.g. adaxial–abaxial axis of cotyledons, Figure 1A). The embryo keeps growing during the torpedo, walking stick and bent cotyledon stages, and a seed (mature embryo) is formed at the end. The mature embryo contains a set of basic organs (shoot apical meristem, root apical meristem, cotyledons) and tissues (epidermis, vasculature, ground tissue). The genetic mechanisms of cell specification and patterning during embryogenesis has been studied in considerable detail in Arabidopsis (reviewed in Ref. 1). While anatomical descriptions and even ultrastructure of Arabidopsis embryos have also been described in some detail, in-depth analysis of cell shapes and subcellular structures, especially in live embryos, has long evaded investigation, likely due to their small size and deep embedding in maternal tissues. In this Short Review, we discuss several publications that have made Arabidopsis embryos more accessible to microscopic observation, and we highlight insights garnered from these.

1.2 | Computational imaging tools for studying cell morphology

Advances in bioimaging protocols and tools have allowed to observe detailed cellular morphology that had until then been invisible. Optical sectioning by confocal laser scanning microscopy enables observing the 3D structure of samples without destroying them. While point scanning microscopes have been widely used over the last decades, multiphoton and light sheet microscopes have become more common in recent years.[2,3] A specific challenge in botanical microscopy is that plant cells contain abundant compounds that produce autofluorescence and different refractive indexes, which disturb microscopic observation. Fixation and clearing of samples have helped to overcome these problems.[4,5] Therefore, the combination of sophisticated sample preparation protocols and advanced microscopes provide multi-dimensional imaging data of various plants. Using such microscopy data, spatial and temporal changes in cell geometry, cell division orientation, cell growth direction and cellular patterns can be visualised and analysed quantitatively. Various softwares have been developed for quantitative analyses.[6,7,8,9] Among these, MorphoGraphX (MGX; www.MorphoGraphX.org) is an open-source software platform for quantitative analysis of cellular morphology and gene/protein expression.[6,7] Visualisation of 3D stacks taken by laser scanning fluorescence microscopy allows the user to freely orient the 3D data set of the sample, which enable to observe detailed morphological features from every direction. For quantitative analysis, 3D data can be projected and summarised on its curved surface. For example, the outline of cells can be projected, and segmented in 3D. The 3D-segmented data can be used for observation of cell division pattern (Figure 1B and C) and also for quantitative analysis such as cell/tissue volume analysis (Figure 2A–C). If the 3D data were obtained from a time-lapse experiment, the same cells can be observed over time by a lineage tracking feature. For example, growth directions and cell proliferation can be analysed by using the time lapse data. Furthermore, cellular gene expression pattern and protein localisation can be projected on the surface to quantify fluorescence

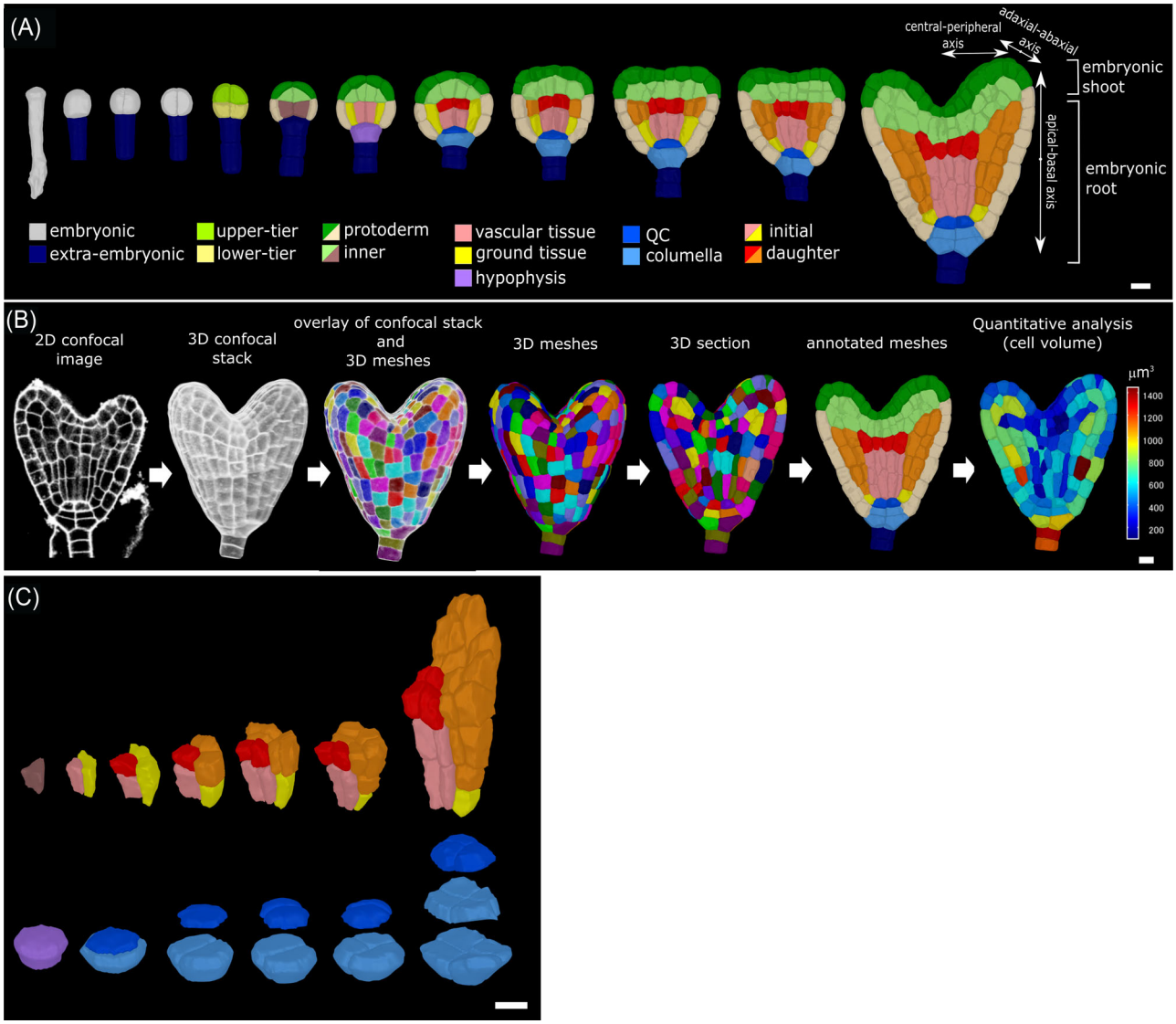

FIGURE 1 Visualisations of 3D cellular morphology of embryos by MorphoGraphX. (A) 3D map of Arabidopsis embryos. From left to right: zygote, 1-cell, 2-cell, 4-cell, 8-cell, 16-cell, early globular, mid globular, late globular, transition, early heart and heart stage embryos are shown. Cells are coloured according to their lineage as shown in the colour legend. Scale bar 10 μm. (B) Analysis of 3D microscopic images by MorphoGraphX. From left to right: 3D confocal stacks are 3D segmented, 3D meshes are annotated based on the cell lineages and quantitatively analysed. Scale bar 10 μm. (C) Cellular patterns of stem cells in the embryonic root from 16-cell stage embryo to heart stage embryo are visualised. Upper row shows pattern of vasculature stem cells and ground tissue stem cells. Bottom row shows pattern of hypophysis, which generates the columella in the root apical meristem. Scale bar 10 μm

signals together with cell geometries. For example, expression profiles of marker genes, orientation of microtubules and localisation of proteins can be quantified. The full 3D analysis can provide complete information of a cell, but requires high resolution images to be segmented.

An exciting application of quantitative imaging data is to feed and test computational simulations. Computational modelling and simulation are ideal methods to solve complex temporal and spatial interactions of genetic networks and cell morphogenesis. Quantitative imaging data sets can be used as modelling templates and provide input to derive parameters to describe the biological system in mathematical terms. Modelling can not only be useful to validate biological hypotheses, but also to discover novel phenomena that were not expected from biological experiments.

1.3 | Quantitative phenotyping of the Arabidopsis embryo

As an example of the power of quantitative imaging and its computational analysis, we established a protocol to image and 3D segment cells of the Arabidopsis embryo.[4]

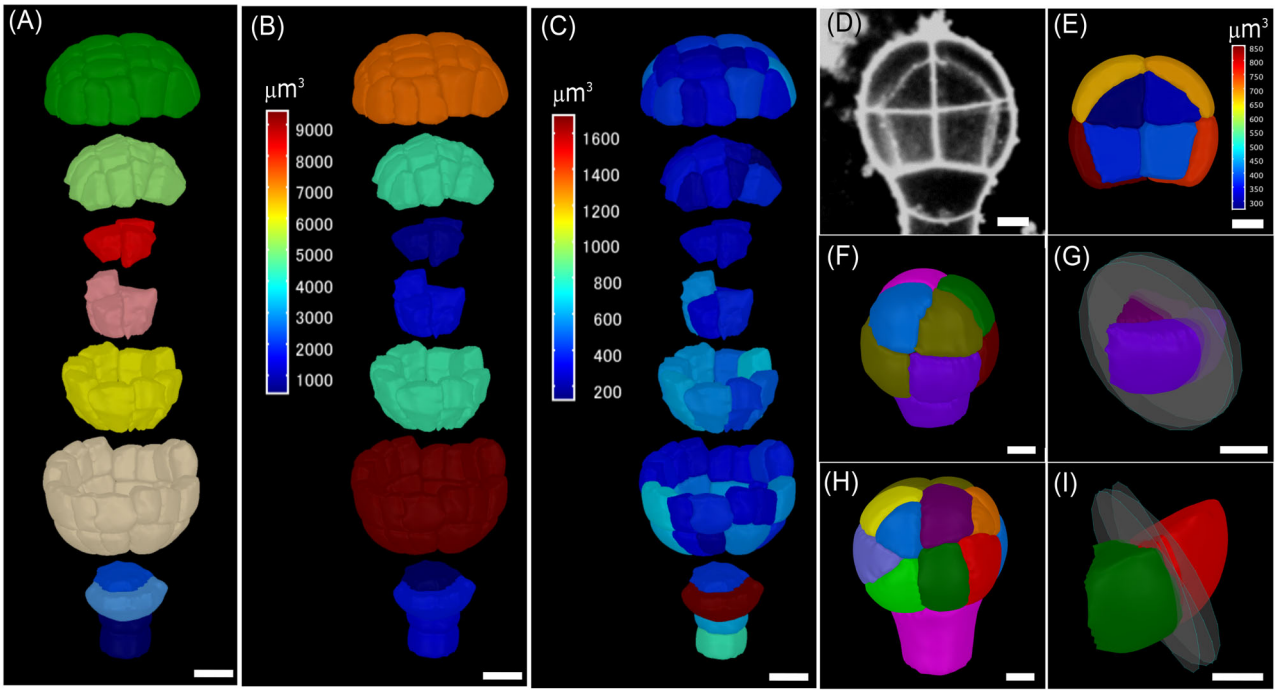

FIGURE 2 Examples of quantitative analyses. (A) Visualisation of morphology, (B) volumes of tissues and (C) cells in globular embryo. (D) In 2D images, inner basal cells look larger than outer basal cells. However, 3D segmented data show that outer cells are larger than inner cells (E). (F–I) Shortest (smallest) wall analysis in wild-type (F, G) and auxin response mutant (H, I) embryos in Arabidopsis. The three possible smallest walls are shown in grey (G, I). Cell division orientation in wild-type embryo does not match with the orientations of smallest walls, whereas in the mutant embryo, cell division orientation matches with the smallest walls. Scale bars 10 μm (A–C) and 5 μm (D–I)

By collecting images from successive stages of embryogenesis, we could derive a 3D lineage map from zygote to heart stage embryos and infer cell division patterns in 3D (Figure 1A–C). Comparison of cell size, embryo size and cell number showed that the first rounds of division occur essentially without growth, while later divisions are accompanied by growth. Cellular volume analysis also identified novel (geometrically) symmetric and asymmetric cell division patterns during embryogenesis. The previous observations of gene expression markers and asymmetric cell division patterns indicated a strong correlation between geometrically asymmetric cell divisions and specifications into different cell identity between the daughter cells generated by the division.[1,4] The volume analysis also illustrated how deceiving 2D information can be. For example, at the 8-cell stage, embryonic cells divide periclinally to generate an inner cell layer and outer cell layer. 2D histological sections would suggest that inner cells have a larger cell volume than outer cells. However, 3D volume analysis showed that outer epidermal cells in fact have a 2.5-fold larger volume (Figure 2D and E). Another surprising finding derived from quantitative imaging is that the earliest divisions in the embryo are aligned with the seed and forecast later symmetries. Two cotyledons are formed in a bilaterally symmetric patterns at the embryonic shoot. Previous studies based on clonal sector analyses suggested that cell lineages generating two cotyledons are already determined at 2- to 4-cell stage embryos.[10] Based on our 3D analysis, we found that the angles of cell division planes at the 2-cell stage are not random, but rather limited to a specific range of angles. We quantified the orientation of the division plane at 2-cell stage embryos, and found that the orientation of the first division plane is orthogonal to the plane of symmetry of the ovule, suggesting that position of cotyledons is determined at a very early stage of embryogenesis.

1.4 | 'Rules' underlying cell division orientation

A key question in plant cell biology is what principle and 'rules' guide the orientation of cell division planes. There are several classical hypotheses, postulated in the late 19th century. Observations led to the generalisation that plant cells divide by creating a new wall perpendicular to the principal direction of growth.[11] It was also assumed that walls are attached at right angles to the exiting walls, and dividing the cell into two equal volumes.[12] The most widely accepted rule was the shortest wall rule proposed by Errera[13] according to which a plant cell divides by creating the shortest wall with constant curvature, like soap

bubbles would. However, because of the lack of an experimental system to examine these rules in 3D, most analyses were done in 2D. We examined this rule in 3D using segmented embryo data sets (Figure 2F–I). We found that at early stages of embryogenesis, embryonic cells divide symmetrically through a division that approximates the 3D equivalent of the shortest wall (minimal surface area). However, between 8-cell to 16-cell stages, cell division deviates from the shortest wall rule (Figure 2F and G), and generates the asymmetric cell division that generates inner and outer cell layers. Imaging, segmentation and computational analysis of mutants in which auxin action was blocked, revealed that auxin response is required to deviate from the minimal surface area rule[4] (Figure 2H and I). Thus, this work demonstrated that embryonic cells divide following the default geometric cell division rule at early stages, whereas this default mechanism is overridden by genetic/hormonal regulation such as activation of auxin signalling.

An independent study[14] used a different computational analysis on a similar data set derived from segmented Arabidopsis embryos. When assuming that new walls are inserted as curved surfaces, as their final shape will be, one can explain even the asymmetric divisions with a single – minimal surface area – rule. However, imaging of live embryos would suggest curvature of inserted walls is limited, and it therefore is an open question which of the two models more accurately reflects reality. It is exciting that these independent studies urge a new question: what is the geometry of newly inserted walls?

1.5 | Tools and approaches for imaging subcellular structures and live embryos

Visualisation is a true enabler of generating new insight into biological processes. However, Arabidopsis embryogenesis has long been relatively inaccessible to high-resolution imaging due to its encapsulation in seed and fruit. Several recent developments have now brought 'light' to early embryogenesis. One challenge had been the observation of subcellular structures in young embryos. By expressing well-established fluorescent markers for subcellular structures from an embryo-specific promoter, ACE lines[15] now allow visualising such structures at high resolution with the help of confocal microscopy. These tools enable new insights, for example in the establishment of cell polarity in the early embryo.

Another key innovation has been the development of protocols for cultivation of Arabidopsis seeds in microfluidics devices for sustained live imaging. Gooh et al.[2] managed to culture fluorescently labelled embryos for a number of cell division rounds, allowing to derive lineage information. When coupled to gene expression analysis and laser-assisted cell ablation, this allowed to demonstrate cell dependencies and regeneration. A challenge still remains to combine high-resolution and live imaging in embryos. Kimata et al.[16,17] have succeeded in this for the earliest steps following fertilisation. They could demonstrate clear patterns of microtubules, F-actin and vacuoles distribution surrounding zygote polarisation and asymmetric division.

1.6 | Where high-resolution imaging and modelling meet

Cell division depends strongly on the cytoskeleton. During cell division, the microtubules associate with the plasma membrane, forming the so-called cortical microtubule array (CMA). The CMA is restricted to a plane that is associated with the nucleus to form the preprophase band (PPB), which forecasts the cell division plane. Now that the microtubule array can be visualised at high spatial resolution, it should become possible to address its role in division plane choice in the embryo. Following observation of the CMA,[15] a computational model was built to simulate microtubule arrangement based on realistic cell shapes and biochemical rules for microtubule behaviour.[18] This led to the abstraction of three principles regulating orientation of CMA. The first principle is built only on cell shape and intrinsic microtubule properties. With these, no realistic cell divisions could be modelled. A second principle modulates the stability of microtubules at cell edges. It has been known that stability of microtubules at the cell edge is affected by the degree of wall curvature. The rate of catastrophe of microtubules (the sudden switch from assembly to disassembly) increases at cell edges with high curvature. However, implementation of the edge catastrophe did not produce wild-type divisions, but could simulate auxin-insensitive cell division. Lastly, a principle was added that auxin-mediated microtubule stability is transiently established at each new division site. The predicted cell division patterns from this final model completely match with cell division patterns in wild-type embryos.

More recently, a detailed investigation of quantitative cellular parameters (polarity, dimensions, aspect ratio, nuclear position) revealed that auxin acts on cell division orientation through its effects on cell shape. Transcriptome analysis of embryos in which auxin response was suppressed showed that the actin and the microtubule cytoskeleton are rather directly influenced by auxin response,[19] which helps rationalising the findings in computational modelling, and offers entry points to mechanistically dissecting the process of division orientation control.

1.7 | Beyond Arabidopsis embryos

While the mechanism regulating the orientation of cell division planes has been studied in the Arabidopsis embryo, it is still unknown if the proposed mechanism also applies to other multicellular organisms. In a recent study, a similar approach of imaging and segmentation was undertaken for the *Brachypodium dystachion* embryo.[20] There the absence of the same degree of division regularity complicates inferences about division rules.

In maize leaf cells, Arabidopsis guard cells and *C. elegans* embryonic cells, cell division orientation was analysed and simulated.[21] First, maize leaf cells with PPB were observed by confocal microscopy, the obtained confocal stacks were segmented and the 3D cell surfaces were imported into the analytical software Surface Evolver.[21] This software predicts the orientation of cell division planes based on the area of new division planes. In maize leaf epidermal cells, the most frequent predicted orientation of division was a transverse-anticlinal division, followed by periclinal division and longitudinal anticlinal divisions. In the majority of cells, localisation of the PPB matched one of these predicted divisions although in a small number of cells, predicted divisions offset from the PPB. This occurred when a PPB or cell wall from a neighbouring cell was adjacent to the predicted divisions, suggesting an active mechanism to avoid creating a four-way junction. To further test the generality of the experimental system, Arabidopsis guard mother cells and *C. elegans* embryonic cells were analysed similarly. As a result, cell division planes of these cells are represented by predictions based on the shortest wall rule.[16] Thus, by simulating the shortest wall, it is possible to predict the future division planes in multiple organisms.

1.8 | Future prospects

Taken together, in this review focusing on the studies of plant embryogenesis, we showcase how genetic tools and computational modelling based on quantitative bioimaging solve questions of developmental biology. While the methodologies discussed here are powerful, there still are possible issues that need to be solved in the future. One challenge is to image for prolonged periods of time at high spatial resolution. Improvements in multi-photon imaging, culturing and microfluidics may help to overcome this challenge. Furthermore, while various tools for quantitative imaging analysis (e.g. 3D segmentation) and modelling are available, it is hard to independently validate the reliability of the 3D data.[22] Lastly, cell segmentation can be laborious, and machine learning can help to automate parts of the process,[8,23] if algorithms are trained on bespoke embryo data sets. With these tools and approaches in place, the early Arabidopsis embryo has the potential to claim its place as a key model in integrating insights into the genetic regulation, cellular execution and implementation of cell division in plants, perhaps including the mechanical aspects of the process.

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ORCID

Saiko Yoshida https://orcid.org/0000-0001-6111-9353

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