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Modelling Meristem Development in Plants

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Running title: Modelling meristem development

Summary

Meristems continually supply new cells for post embryonic plant development and coordinate the initiation of new organs such as leaves and flowers. Meristem function is regulated by a large and interconnected dynamical system that includes transcriptional networks, intercellular protein signalling, polarized transport of hormones and a constantly changing cellular topology. Mathematical modelling, in which the dynamics of a system are simulated using explicitly defined interactions, can serve as a powerful tool for examining the expected behaviour of such a system given our present knowledge and assumptions. Modelling can also help to investigate new hypotheses *in silico* both to validate ideas and to obtain inspiration for new experiments. Several recent studies use new molecular data together with modelling and computational techniques to investigate meristem function.

Introduction

Due to the often regular and symmetrical patterns generated by plants, plant architecture has not only fascinated biologists but also mathematicians and artists alike for centuries [1]. However over the past couple of years mathematical and modelling approaches to understanding plant development have gained fresh momentum [2,3], partially due to inexpensive computing power but also due to the rapid increase in detailed molecular data related to plant development. The origin of many plant developmental patterns can be traced back to meristems located at the growing tips of roots and shoots, from which most postembryonic structures are derived. In this review we will focus on how modelling and computational techniques have recently been combined with detailed molecular data to help understand meristem development and function.

Meristem maintenance

One of the astounding features of meristems is their ability to maintain a specific cellular structure and growth pattern throughout the lifetime of the plant, which can in some cases last thousands of years. Experimental studies have shown that shoot apical meristem (SAM) size, in particular the region of undifferentiated cells at the very apex, is regulated via a negative feedback loop between the transcription factor WUSCHEL (WUS) and a small secreted peptide CLAVATA3 (CLV3). *WUS* is expressed in the organizing centre, a small subapical region in the interior of the shoot. It promotes stem cell identity and positively regulates *CLV3* transcription. CLV3 in turn acts together with the receptor kinase CLAVATA1 to repress *WUS* expression, thus creating a negative feedback loop [4,5,6,7,8]. Although this feedback loop provides an intuitively simple system for regulating the size of the *WUS* expression domain, and hence proliferation of stem cells in the SAM, it leaves several questions open, including the question of how the pattern of the *WUS* expression domain is specified.

The *WUS* expression pattern is intriguing since it is capable of reorganising itself after disruption by laser ablation [9], mirroring the ability of meristems in general to self-

organize after wounding . Self-organization is also a property exhibited by reaction-diffusion systems as proposed by Turing and Meinhardt [10,11]. As a test to see how well such a scheme might work to control *WUS* expression, Jönsson et al (2005) examined a model in which *WUS* expression was placed under the control of a reaction-diffusion mechanism [••12]. The model was simulated on a two-dimensional cellular template extracted from a transverse confocal microscope section of the SAM centre (Figure 1a-c). The model created one maximum of *WUS* expression within the meristem region, which was centred within the tissue using a hypothetical repressive signal emanating from the epidermal, or L1, cell layer. Model parameters were tuned by comparing the output of the model directly with real *WUS* expression, also extracted from the template data (Figure 1d). When the central cells expressing WUS were removed to simulate the effect of laser ablation, *WUS* expression reappeared on either side of the ablated region in a similar fashion to the observed experimental response. An alternative model using constitutive expression modulated by the repressive signal from the L1 failed to re-organize *WUS* expression in this way. Although this model is abstracted from the biology to a considerable degree, it suggests that the basic mechanism of long-range inhibition together with local reinforcement may account for the self-organizational properties of not only *WUS* expression but also meristems in general. While CLV3 is a prime candidate for a WUS-induced long-range inhibitor, recent experiments also show that members of the HD-ZIP class of transcription factors play a repressive role in regulating *WUS* expression [13,14]. It will be interesting to test whether the expression of these genes is, like *CLV3*, also dependant on WUS activity and whether models can be used for discriminating between different scenarios of long-range inhibition.

How is auxin distributed in meristems?

Auxin flow within the root meristem is coordinated via multiple members of the PIN family of auxin efflux mediators as well as the auxin influx mediator AUX1 [•15,16]. In Swarup et al. (2005) experiments and modelling were combined to investigate the role of auxin in mediating gravitropism [••17]. A three dimensional model corresponding to the elongation zone proximal to the meristem was constructed using the stereotypical arrangement of root cells as well as detailed distribution patterns of PIN1, PIN2, and AUX1 (Figure 2a). The model used these data together with the chemiosmotic

transport theory [18,19] to show that *AUX1* expression in the epidermal cells should be sufficient to mediate apical transport of auxin from the root tip with only moderate diffusion. Given an asymmetric pulse of auxin from the root apex in response to an altered gravity vector, epidermal cells are predicted to maintain this asymmetry throughout the elongation zone in order to promote differential growth. In fact the authors go on to predict that PIN2 should not be required in the epidermis for this transport function since weak PIN1 in these cells should suffice. Instead PIN2 is suggested to be only necessary for auxin efflux into the epidermis from the lateral root cap. This remains an untested prediction from the paper. This study represents an important step towards modelling the flux of auxin throughout the root meristem and it would be interesting to see whether such a complete model might account for the specific auxin-induced expression and protein degradation patterns of the various PIN proteins.

In the SAM, PIN1 expression and localization in the epidermal layer appears to be important for determining the auxin distribution in relation to where new primordia are formed [20, •21] (see below). However PIN1 localization patterns in these cells are harder to interpret compared to the root. Some cells are not clearly polarized and PIN localization patterns change constantly as primordia development proceeds around the meristem periphery [•21]. To try to deduce auxin distribution patterns in these cells Barbier de Reuille et al. (2006) used confocal imaging to visualize and document PIN1 immunolocalization patterns in the L1 [••22] (Figure 2b). After hand-marking these patterns such that each side of each cell was designated to either contribute or not contribute transport to the adjoining cell they introduced a simplified model for PIN1 dependant auxin flow (not based on the chemiosmotic transport mechanism) that included diffusion. Encouragingly their simulations predicted auxin peaks at positions where new primordia were about to form, as has been shown to occur experimentally. Unexpectedly, their model also predicted high auxin levels at the shoot apex (Figure 2b). In support of this prediction the authors were able to successfully detect IAA specifically in the meristem apex using an IAA specific monoclonal antibody and Gas chromatography-Mass spectrometry (GCMS). By also showing that the synthetic auxin reporter DR5 was not sensitive to exogenous auxin in this region the authors concluded that perception of auxin is suppressed at the level of primary auxin response genes at the

meristem centre (however contradictory results are presented in [••23]). Lastly, the simulations of their flow model lead the authors to hypothesize that auxin levels increase at new primordial positions because of over accumulation of auxin in the meristem centre and depletion of auxin by neighbouring primordia. However the question of how PIN1 polarity is coordinated to produce this flow pattern is left open (see below).

The heart of phyllotaxis – an auxin mediated spacing mechanism

The positioning of lateral organs (phyllotaxis) has long been the subject of modelling studies. One of the key findings from these studies is that many, if not most, of the complex patterns of organs observed in nature can be generated by any kind of regular spacing mechanism combined with a gradually expanding generative region such as a meristem growing over time [24,25,26,27,28]. Recent experimental studies have shown that an essential part of the mechanism involves the transport of auxin, via the asymmetrically localized PIN1 auxin efflux carrier, to positions where primordia are destined to form [29,30,31]. Thus a central question is what coordinates PIN1 localization in such a way? Two recent studies have proposed a mechanism that is not only capable of generating close to observed PIN1 localization patterns but is also able to spontaneously generate regularly spaced peaks and troughs of auxin concentration [••23, ••32]. At the core of these models is a feedback system in which PIN1 protein gets localized to the membranes of a cell closest to neighbouring cells that contain the most auxin. Thus cells with high auxin content polarize their neighbours towards them, further increasing their auxin content until the flux due to polar transport is balanced by diffusion.

Although both models are based on the same hypothesis for PIN polarization, they differ in their approaches. Smith et al do not always use equations that are easily interpretable in terms of biochemical mechanisms and to stabilize their patterns they include additional rules for localizing PIN1 that abstractly relate to primordial differentiation. However their model parameters are tuned by comparing the resulting phyllotactic patterns with experimental data from *Arabidopsis*, and a good correspondence is achieved. Lastly, their model is run on a global growth template defined by the authors to resemble real meristem tissue growth (Figure 3a). In contrast, Jönsson et al create a dynamic cellular template using spring mechanics to model growth.

Although such a growth model does not appear very "plant-like" it represents an early attempt to couple real cell wall mechanics to a signaling and gene regulatory model (Figure 3b). The auxin transport model is also defined differently using mechanistic equations, and an experimental template is used together with the chemiosmotic auxin transport theory to estimate parameter values for modeling PIN1 polarization. This model is also capable of generating phyllotactic patterns, although not as stably as those generated by the Smith et al model. Overall, the picture that emerges is that even if true, PIN1 polarization by cytoplasmic auxin concentrations in neighboring cells can only be part of the story. Perhaps this is not surprising given the existence of many other players such as the PID kinase [33], *SHOOTMERISTEMLESS* (*STM*) and *CUP-SHAPED COTYLEDONS*1/2 (*CUC*1/2) which also mediate organ positioning and growth [34]. Nevertheless these initial models provide us with a novel potential positioning mechanism based on up-to-date molecular data and hypotheses that are experimentally testable.

Integration of gene regulatory models with morphogenesis and mechanics

So far we have been discussing discrete models that deal with gene expression patterns and the distribution of signalling molecules within "virtual" cellular templates. Each template has been different, from the static 3D root architecture of Swarup et al. to the mechanically driven 2D template from Jönsson et al. Ideally the model template should resemble the real plant tissue as closely as possible and such a template may not only involve creating a cellular architecture of the tissue under study but may also involve the use of gene expression patterns, protein distributions and the time-evolution of these data. This is where confocal live-imaging techniques potentially offer significant new advantages for assessing and developing plant developmental simulations. In deReuille et al. a novel semi-automatic protocol for obtaining the cellular architecture of the meristem epidermal layer is presented [••35]. The procedure is based on segmenting epidermal cell outlines using a membrane localized fluorescent dye or GFP imaged using confocal microscopy (Figure 4a and b). From these data the neighbour relationships can be determined and by conducting time-lapse imaging, the shapes of these cells can be extracted over time (Figure 4c). Although this type of data has also been obtained using a simpler non-invasive replica method [36,37,38], this study potentially represents a first

step towards using confocal imaging to extract a multivariable modelling template that includes gene expression patterns and protein localization data along with cell shape dynamics.

Conclusion

It is an exciting time for understanding the role of auxin in meristems and this is reflected in the choice of papers we have focused on in this review. The use of new imaging and perturbation techniques is also providing us with data for use in models in unprecedented detail [•21,39,•40,•41,•42]. In fact, we feel that these new experimental techniques are of great help in providing the detailed dynamic data required for inspiring mechanistic models as well as for testing the models adequately.

A challenge not yet addressed in the literature is to link gene regulatory models with realistic models for cell wall mechanics since only then can models bridge the gap between signalling and morphogenesis. This may not be a trivial task, given the anisotropic and visco-elastic nature of plant cell walls [43]. Another future challenge, as more and more data becomes available, is to somehow integrate and combine models and hopefully this is where efforts at creating standardized languages such as Systems Biology Markup Language (SBML) may bear fruit [44].

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The authors discriminate among different models for how a sharp domain of WUS expression can be maintained by comparing directly with WUS extracted from an experimental template as well as with perturbation experiments. This paper provides an early example of the potential of direct integration of modelling techniques and confocal data.

13. Prigge MJ, Otsuga D, Alonso JM, Ecker JR, Drews GN, Clark SE: **Class III homeodomain-leucine zipper gene family members have overlapping, antagonistic, and distinct roles in Arabidopsis development**. *Plant Cell* 2005, **17**:61-76.
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•15. Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, Friml J, Heidstra R, Aida M, Palme K, Scheres B: **The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots**. *Nature* 2005, **433**:39-44.

Detailed expression and polarization data for five PIN proteins is presented, which illuminates the auxin flow within the root meristem. In addition several multiple mutants are investigated and interactions with the PLETHORA root stem cell specification genes are shown. This paper provides detailed data for a module for root meristem maintenance, integrating auxin, polarized transport and transcription factors.

16. Swarup R, Friml J, Marchant A, Ljung K, Sandberg G, Palme K, Bennett M: **Localization of the auxin permease AUX1 suggests two functionally distinct hormone transport pathways operate in the Arabidopsis root apex**. *Genes Dev* 2001, **15**:2648-2653.
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The authors combine modelling and tissue specific induction of the AUX1 auxin influx mediator to show which cell types are important for mediating the root gravitropic response. The presented model use detailed information of the polarization of the auxin transport mediators as input and use the chemiosmotic transport theory with quantitative estimates for parameter values, which results in a model capable of providing quantitative predictions for the auxin flow and distribution in the root elongation zone, both in wild type and during gravitropic response.

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This paper gives a broad overview temporal and spatial changes to gene expression patterns that occur during primordium development on the *Arabidopsis* inflorescence meristem. Correlations between gene expression changes and changes to PIN1 polarity patterns suggest various causal hypotheses that can be investigated both by modelling and in future experiments.

••22. de Reuille PB, Bohn-Courseau I, Ljung K, Morin H, Carraro N, Godin C, Traas J: **Computer simulations reveal properties of the cell-cell signaling network at the shoot apex in Arabidopsis**. *Proc Natl Acad Sci U S A* 2006, **103**:1627-1632.

The complex PIN1 polarization pattern is manually extracted for the epidermal cells at the SAM from confocal data in multiple plants. This polarization data is used as an input to an auxin transport model to give an estimate of the auxin distribution in the SAM. The model is verifying high auxin concentrations at sites of new primordium initiation and, equally important, predicts high auxin levels at the apex.

••23. 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.

This paper (and ref. [••32]) investigates a novel hypothesis for a global spacing mechanism based on local cell-cell interactions and in accordance with current data on auxin transport and PIN polarization. Simulations on a 2D growing meristem template show that the model is potent to drive phyllotactic patterning. Together with [••32] this work represents a first attempt to bridge the gap between the long history of models for phyllotaxis and molecular biology.

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See comment on ref [••23].

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••35. de Reuille PB, Bohn-Courseau I, Godin C, Traas J: **A protocol to analyse cellular dynamics during plant development**. *Plant Journal* 2005, **44**:1045-1053.

This paper describes a computer-assisted protocol for segmenting the cells that make up the SAM epidermis. By repeating this procedure over time the authors are also able to measure growth rates. Future improvements on this method may enable the extraction of a dynamic three dimensional template that could contain information for gene expression and protein localization in addition to cell architecture.

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Using dexamethasone (Dex) -induced double-stranded RNA interference, the authors assess the immediate consequences of the loss of CLV3 function to *Arabidopsis* inflorescence meristems using confocal microscopy. The expression of GFP under the control of the *CLV3* promoter is used as a marker for central zone identity while cell divisions were tracked using a membrane localized yellow fluorescent protein. The authors found that upon treatment with Dex the number of cells expressing GFP (under the CLV3 promoter) increased and this was later followed by an increase of the SAM size, followed again by an enlargement of the *CLV3* expression domain. These observations show that peripheral zone cells get re-specified as central zone cells when CLV3 function is compromised and this response can be separated temporally from the increased number of cell divisions that give rise to the enlargement of the meristem. This study provides an example of the type of detailed data required to formulate realistic models of meristem function.

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The authors used an ethanol-inducible gene expression system to over-activate *CLV3* in its normal expression domain in the inflorescence meristem. While a sustained CLV3 induction caused termination of shoot meristem development, a pulsed induction led to a rapid but transient repression of *WUS* expression. Both *WUS* and endogenous *CLV3* expression were measured following the pulsed CLV3 induction using quantitative RT-PCR. In addition they helped to characterize the non-linearity and robustness of the CLV-WUS feedback loop by showing that the CLV3 signal strength can vary tenfold without disrupting shoot meristem development. This paper gives quantitative insight into the dynamics of the CLV3-WUS feedback loop.

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This study documents dynamic changes in auxin transport patterns and cell type patterning that occur during regeneration of the *Arabidopsis* root tip after laser ablation. Using time-lapse confocal imaging the authors find that changes to the auxin distribution occur rapidly, followed by changes to cell identity, which in turn is followed by changes

to PIN polarity patterns. The *PLETHORA* genes as well as *SHORTROOT* and *SCARECROW* are required for proper re-patterning to occur. By dissecting this process in detail, the authors are able place auxin, cell fate patterning and polar auxin transport into a linear pathway.

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Figure legends

Figure 1. Segmentation of 2D cell geometry and *WUS* expression from confocal data

(a) Original transverse confocal optical section through the *Arabidopsis* inflorescence meristem. The cell membranes (red) are stained with FM4-64. Endoplasmic reticulum localized GFP under the control of the *WUS* promoter is also shown (green). **(b-c)** Cells extracted from the membrane data by removing the background (b), and using a watershed-like algorithm for segmentation (c). **(d)** Quantitative estimation of WUS expression from GFP intensities in segmented cells. Modified from [••12] with permission from the National Academy of Sciences, Copyright 2004.

Figure 2. Output from auxin transport models of phyllotaxis.

(a) Whorled pattern of auxin maxima (light green) and PIN1 distribution (red) generated by the model proposed by Smith et al. (2006) on expanding two-dimensional template mimicking the meristem epidermis. Modified from [••23] with permission from National Academy of Sciences, Copyright 2006.

(b) Spiral pattern of auxin maxima (red and orange) generated by the model proposed by Jönsson et al. (2006) on mechanically growing layer of cells mimicking the meristem epidermis. Modified from [••32] with permission from National Academy of Sciences, Copyright 2006.

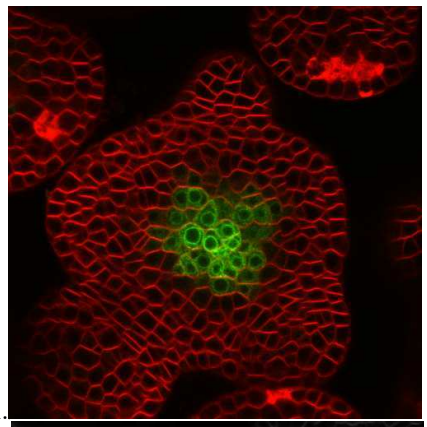
Figure 3. Modelled auxin distribution pattern in the root and shoot

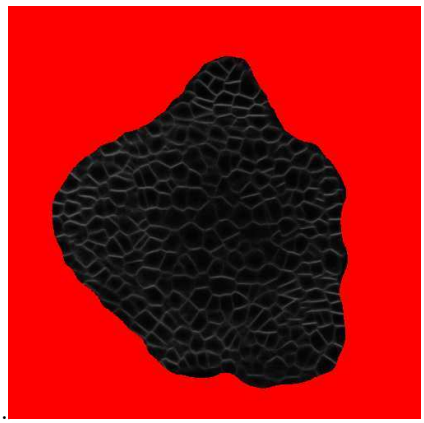
(a) Illustration of the cylindrical template representing the root elongation zone used in Swurap et al. (2006). The 3D model incorporates the epidermis, cortex, and endodermis cells and auxin transport mediator locations illustrated in the inset (pink PIN1/2, orange AUX1). The blue shades illustrate auxin concentrations in the epidermis when auxin is deposited at the lower side from the lateral root cap (arrow) (Adapted with permission from Macmillan Publishers Ltd: Nature Cell Biology [••17], Copyright 2006. **(b)** Transverse section of the SAM showing anti-PIN1 immunolabeling (top) and simulation output showing auxin concentrations (bottom) from Barbier et al. (2006). The cells at the location of a new primordia (circle) as well as the apex have high auxin

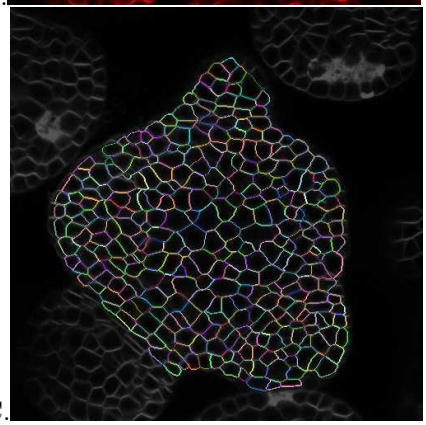
concentrations. Modified from [••22] with permission from National Academy of Sciences, Copyright 2006.

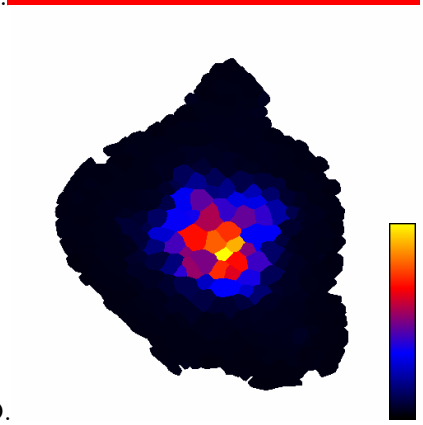
Figure 4. 4D segmentation of the shoot meristem epidermis

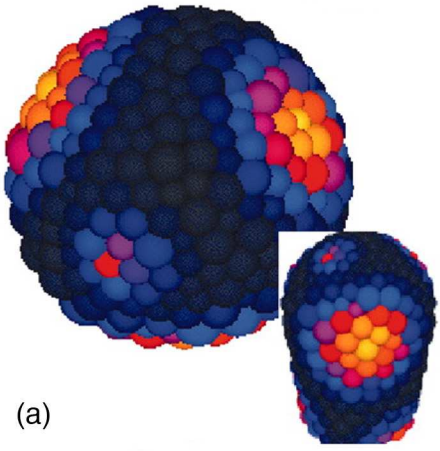
(a) Top view of the meristem epidermis extracted from a stack of transverse confocal images where the background has been removed and the cells manually extracted. (b) Three dimensional view of the epidermis of the meristem. (c) Vertex movements as calculated from two consecutive time-points of the surface reconstruction. Note also new cell walls marked in blue. Modified from [••35] with permission from Blackwell Publishing Ltd, Copyright 2005.

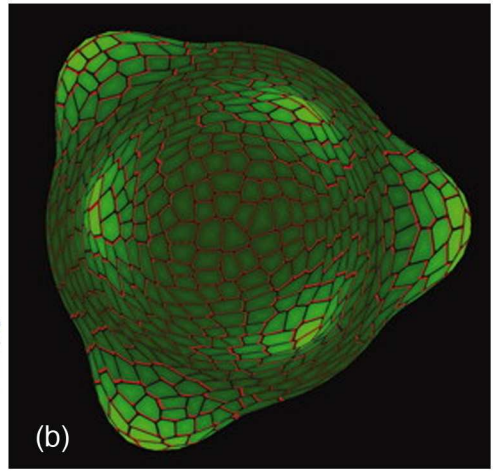
A. 

B. 

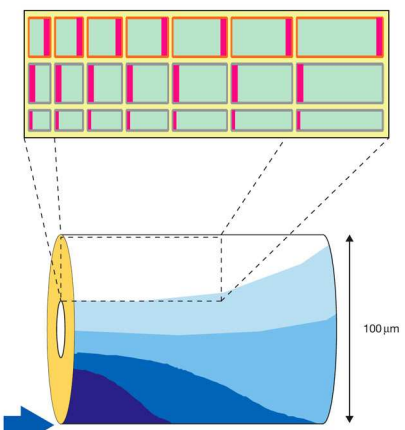
C. 

D. 


(a)


(b)

(a)



(b)