Developmental Modeling of the *Arabidopsis* Shoot Apical Meristem

http://www.computableplant.org

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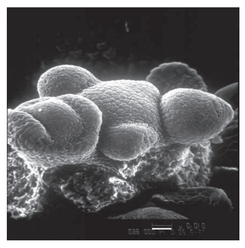
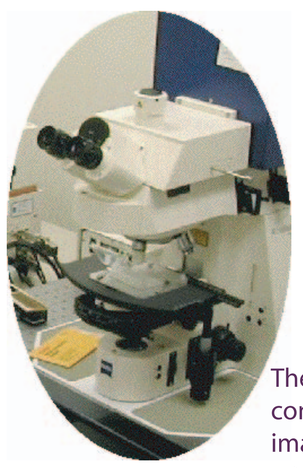
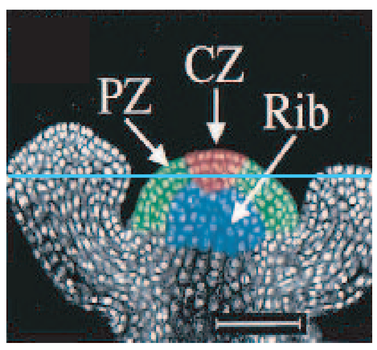
The Computable Plant

The computable plant project is developing an end-to-end research and modeling framework for the *Arabidopsis* SAM (shoot apical meristem). We observe several cell type specific markers for growth and differentiation in real-time in live plants with a dedicated confocal laser scanning microscope. Using a combination of computational modeling and image processing techniques we then infer specific transduction pathway data and fit mathematical models to produce two- and three- dimensional visualizations of the growing SAM, include phyllotactic and leaf-vein development. In particular, we have developed several GFP (Green Fluorescent Protein) variants that allow us to observe various meristerm and floral primordial features including both cell walls and cell nuclei as well as track specific cell lineages over time. Our aim is to determine the spatial and temporal relationships between different genes in an effort to understand how primordial cells are progressively specified. These markers will allow us to correlate gene expression changes with cell growth over time. Thus far the live imaging technique has led to the development of a spatial and temporal map of cell division patterns. We have observed that primordium development is a sequential process linked to distinct cellular behavior, and that the amount of cell division is comparable in regions of the SAM where successive primordia arise. Changes in cell division orientation are associated with initial outgrowth of a flower primordium. These changes are followed by a rapid burst of cell expansion and cell division that transforms a flower primordium into a flower bud. We are developing software to automatically extract cell positions from time-lapse observations; integrating this information with inferred pathways, it should be possible to produce forward simulations that predict and visualize meristem growth.



These three views of *Arabidopsis thaliana* illustrate some of the scales at which developmental processes are seen. In the image below, the SAM is stained with propidium iodide to show nuclei, and colored to show cytological regions: the Central Zone (CZ, red) at the tip; the surrounding peripheral zone (PZ, green); and the deeper Rib meristem (blue). Cell division occurs rapidly in the PZ and Rib but more slowly in the CZ. Continued cell division the entire SAM to move upward, with central cells of the rib differentiating into the plant stem. Leaves and flowers form in specific regions of the PZ. Epidermal cell layers (L1 and L2) divide anticlinally, i.e, new cell walls form perpendicularly to the SAM surface, while deeper cells divide in any direction. Scale bar: 50 microns [Meyerowitz 1997].





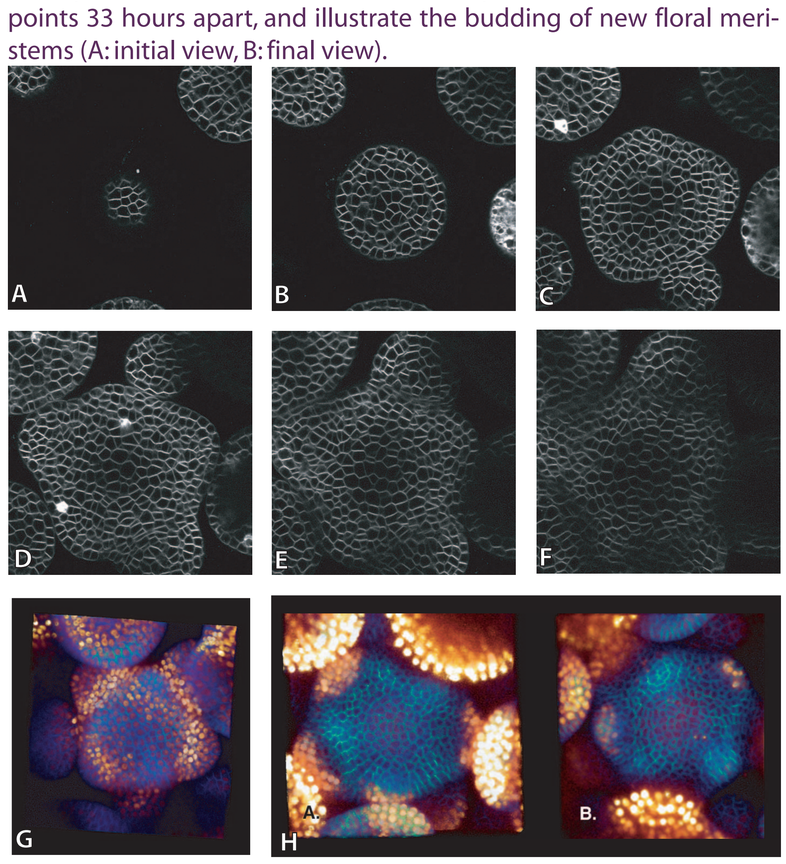
The Zeiss LSM 510 meta upright scanning confocal microscope used to obtain 3D images of live plants (left). The location of a typical cross section is indicated on the image above.

Educational Outreach

We have developed a new set of techniques for high school, pre-service science teachers, and undergraduate students through our partnership with the Huntington Botanical Gardens in San Marino, CA. Outreach activities culminate in a summer institute for high school students and a summer workshop and follow-up winter institute for high-school biology teachers "The ABCs of Developmental Botany: Integrating Plants into the Classroom." Activities take place at the Huntington, which hosts over 500,000 visitors each year.

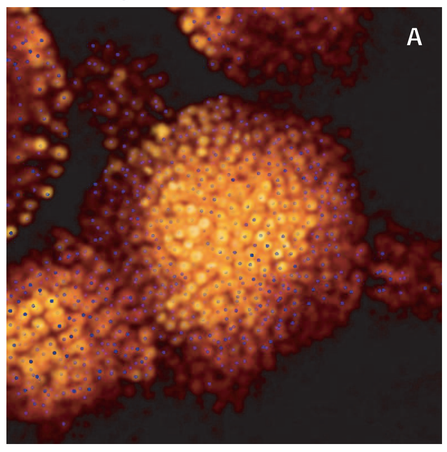
How The Images Stack Up

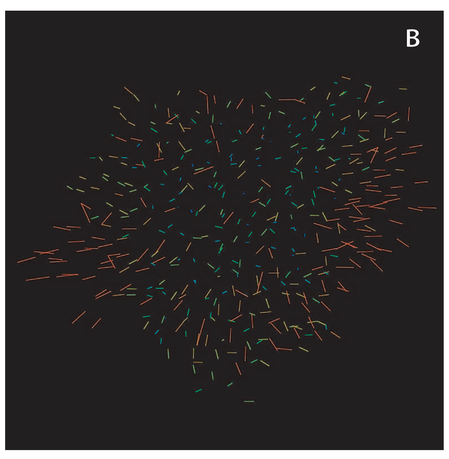
The 3-dimensional reconstruction starts from "stacks" of horizontal cross sections. (A-F) Every 10th section in a set of 51 plasma-membrane dye images (FM4-64). Horizontal resolution is 0.15 μ and the entire stack spans 43μ. The images are 150μ on a side. While these dyes highlight the membrane, other constructs allow visualization of the nucleus, the ER, and especially, specific proteins relevant to SAM development and differentiation. (G) PIN1GFP in combination with pCUC:3XVENUS N7, illustrating how two different proteins can be visualized at the same time. (H) The two images at the bottom right show pPIN1:PIN1GFP in combination with pFIL: dsRED N7 at two time points 33 hours apart, and illustrate the budding of new floral meristems (A: initial view, B: final view).



In any 3D image stack there is a correspondance problem: which cells in one image correspond to which cells in the adjacent cross-section? Cell walls that are transverse to the image are clearly visible, but it is possible to miss nearly horizontal walls that lie between sections and must be inferred.

With a time-course of 3D stacks the formation of floral meristems, cell growth, movement, and division all complicate the problem. Cell motion due to plant growth is illustrated in the two images below. The top image (A) shows GFP labelled Histone 2B protein and the corresponding nuclear locations, inferred from a gradient descent-based algorithm on image intensity.

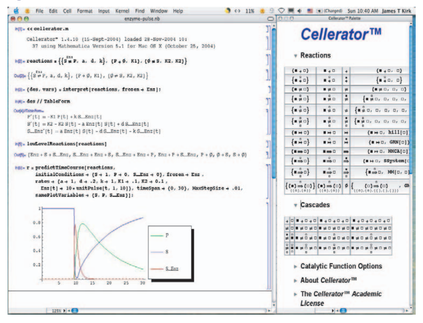




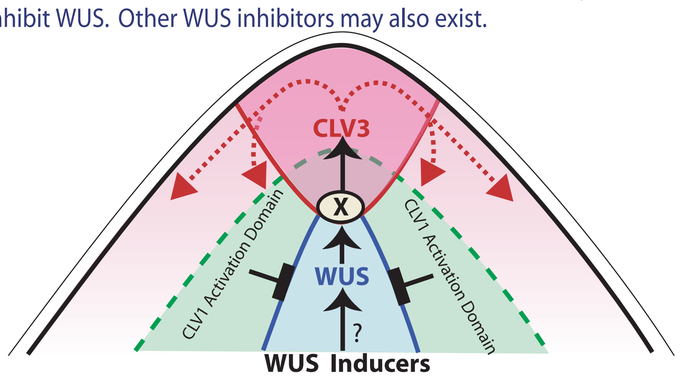
To obtain image (B), a series of such images were taken over 18 hours. Nuclei positions were extracted and then the correspondence was found for those nuclei that did not divide using a Softassign algorithm [Rangarajan, 1996]. The positions were rigidly aligned using the correspondence and least squares and then the difference vectors generated and displayed. The colors were assigned to line segments based on the magnitude of the displacement. Image size: 150μ on a side.

Modeling and Simulation

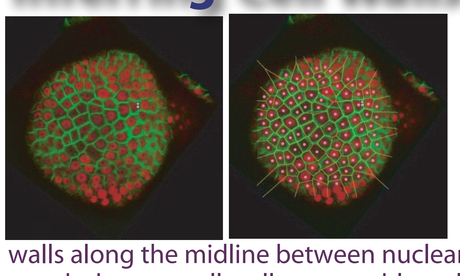
Cellerator is used to design signal transduction networks (STN) based on known or hypothesized biochemical interactions and gene regulation. Input to *Cellerator* is via a set of arrow-based reactions representing the network; *Cellerator* translates the STN into a system of differential equations. An automated code generator reads the Cellerator SBML model and generates and links an efficient simulation engine. Spatial dynamics are described by a network of point localized cells joined by "spring" dynamics. Ellipsoidal and polyhedral cell models with stress/strain tensors are being developed.



A model based on gene expression imagery of key regulatory proteins in the SAM's development is illustrated below. WUSCHEL (WUS), a homeodomain transcription factor expressed in the Rib meristem activates an unknown protein, X, which in turn induces CLAVATA 3 (CLV3) in the subepidermal layer. CLV3 is believed to be the ligand for CLAVATA1 (CLV1), a receptor kinase, that in turn is hypothesized to inhibit WUS. Other WUS inhibitors may also exist.



Inferring Cell Walls



Inferring the locations of cell walls is necessary to model intercellular connectivity and produce meaningful visualizations. The Voronoi diagram - a construction from computer science - places cell walls along the midline between nuclear centers. The Voronoi cells match the true cell walls reasonably well in this pair of images. Do cell walls naturally place themselves in this way for optimal resource distribution, or is this a coincidence? The quality of the match could also be a result of human visual perception and the fact that most of the cells in this image are roughly equal in size.

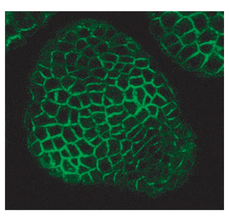
Phyllotaxis

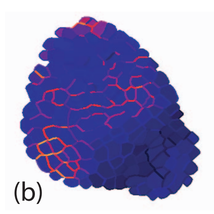
New plant organs form at regular intervals and angular separation as the plant grows. Two major molecular determinants are auxin (indole-3-acetic acid) and PIN1, a membrane protein that may be an auxin receptor or membrane transporter: phyllotaxis fails in PIN1 mutants and can be induced by point auxin application to normal meristems. PIN1 polarization to cell walls may be induced by auxin, as for example in this model:

$$P_i \xrightarrow{f(a_j)} P_{ij} \quad \text{and} \quad \frac{da_i}{dt} = K_p - K_d a_i - T a_i \sum_j^{N_i} P_{ij} + T \sum_j^{N_i} a_j P_{ji} + D\left(\sum_j^{N_i} a_j - N_i a_i\right)$$

Transport *Diffusion*

In the first equation PIN1 is polarized to the membrane by auxin; the other describes auxin flux, transport and diffusion. GFP-labelled PIN1 (a) and the corresponding simulated auxin concentrations (b) are shown here on the right.





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