A parametric active 4D model for the automatic construction of developmental atlases from confocal images of shoot apical meristems

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Understanding phyllotaxis by building a quantitative atlas of SAM development

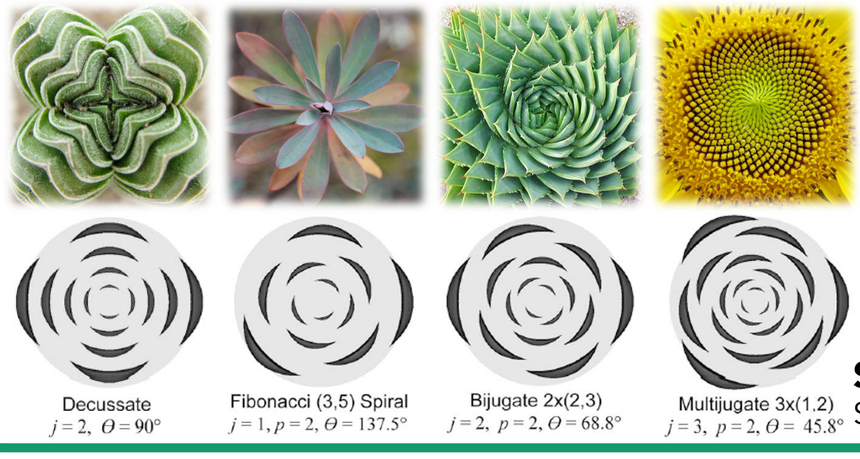
A parametric active 4D model for the automatic construction of developmental atlases from confocal images of shoot apical meristems

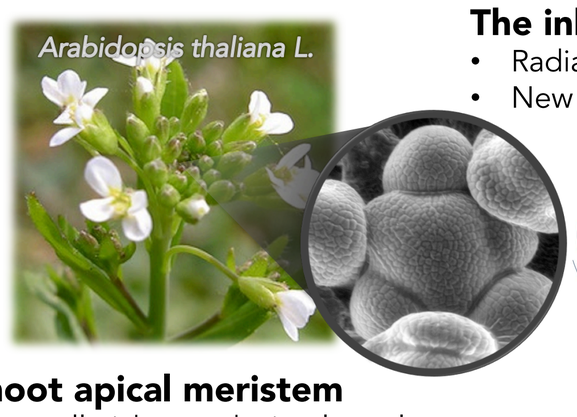
Guillaume Cerutti [a,b,☆], Carlos Galvan-Ampudia [b], Jonathan Legrand [b], Teva Vernoux [b], Christophe Godin [a]

a : Virtual Plants INRIA Project-Team, joint with INRA and CIRAD, Montpellier, France.

b : Laboratory for Reproduction and Development of Plants, Université de Lyon, ENS-Lyon, INRA, CNRS, Lyon, France.

Phyllotaxis as self-organized pattering of organs at the shoot apical meristem: the Inhibitory Field theory

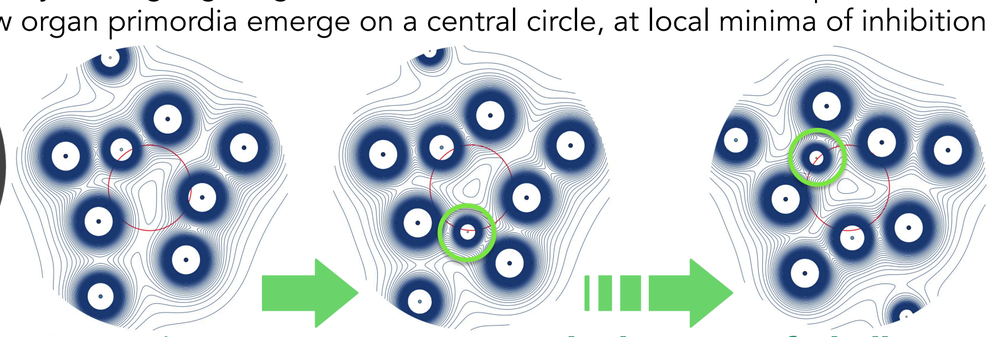




Shoot apical meristem
Stem cell niche producing lateral organs

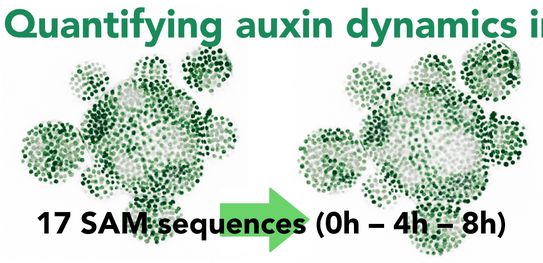
The inhibitory field model of phyllotaxis *(Douady & Couder, 1996)*

- Radially moving organs generate an inhibition that sums into a spatial field
- New organ primordia emerge on a central circle, at local minima of inhibition



Center/Organ size ratio controls the type of phyllotaxis

Quantifying auxin dynamics in the SAM to understand patterning



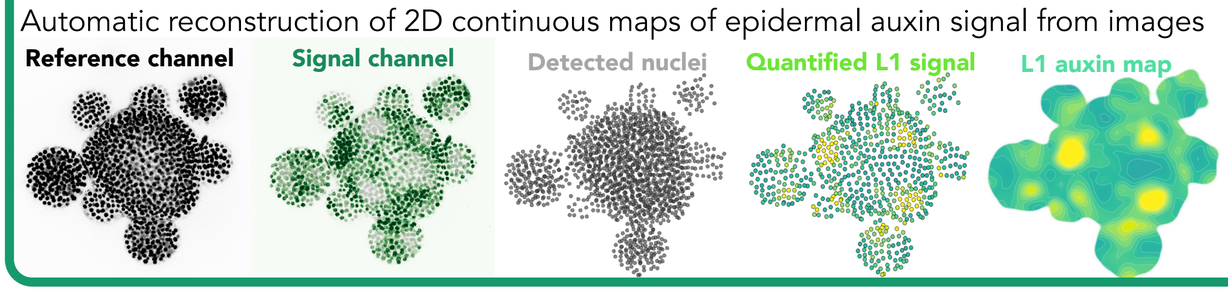
17 SAM sequences (0h – 4h – 8h)

Auxin (hormonal signal)
Natural candidate for biological inhibition

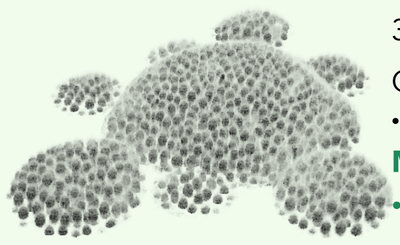
- Needed to trigger organogenesis
- Depleted by transport around organs

Time-lapse confocal imaging of ratiometric negative auxin reporter **r2DII** *(Weijers, 2014)*

Automatic reconstruction of 2D continuous maps of epidermal auxin signal from images



Automatic 3D nuclei detection and signal quantification pipeline



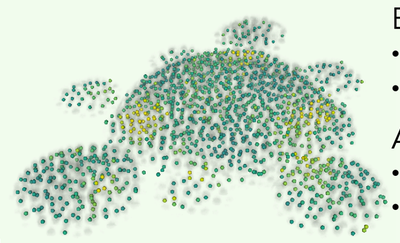
3D confocal microscopy image stacks (0.5x0.5x1µm voxels)

Cell nuclei detected as spherical blobs of typical size

- Local maxima in 3D Gaussian scale-space (σ from 0.8 to 1.4µm)

Method evaluated on expertized SAM images

- **Precision > 0.98, Recall > 0.95**

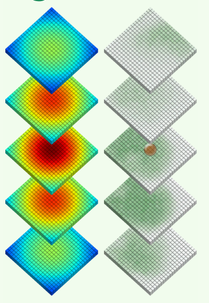


Estimate signal intensity on nuclei points

- Gaussian-weighted average of image intensity
- Ratiometric quantification Signal/Reference

Automatic labelling of L1 nuclei

- Epidermis (implicit) surface mesh reconstruction
- Distance-based selection of detected nuclei



How to aggregate all the information from different time sequences into a single developmental atlas?

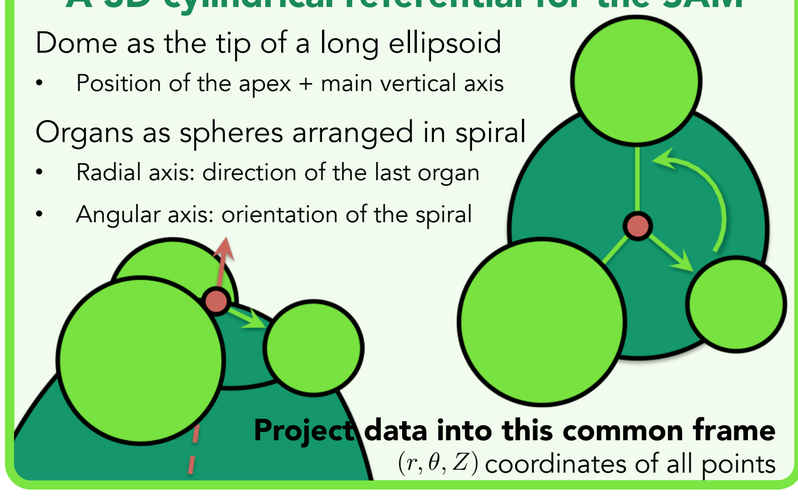
A 3D cylindrical referential for the SAM

Dome as the tip of a long ellipsoid

- Position of the apex + main vertical axis

Organs as spheres arranged in spiral

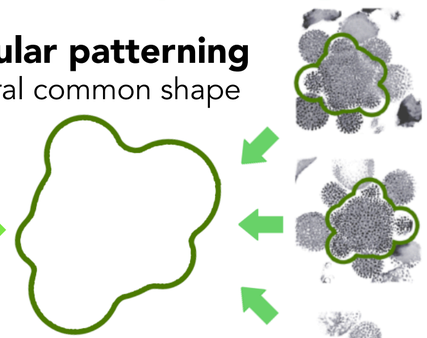
- Radial axis: direction of the last organ
- Angular axis: orientation of the spiral



Project data into this common frame
(r, θ, Z) coordinates of all points

Spatio-temporal registration method: fitting a 4D parametric SAM shape model

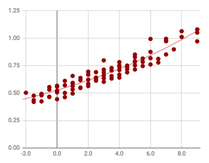
Regular patterning
Natural common shape



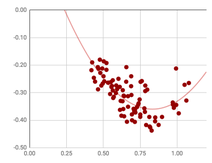
Spiral phyllotaxis
Spatio-temporal periodicity

Phyllotaxis-based meristem shape model with few parameters

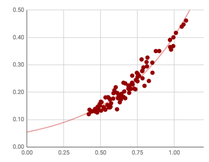
- **6 parameters** for the dome, **2 parameters** for the organ referential
- **1 developmental time parameter** controls organ parameters (r, θ, Z, R)
- Assuming regular "golden" divergence angle: $\theta_{i+1} = \theta_i + o \cdot \psi$
- **Time periodicity of 1 plastochron** (gap between consecutive organs)
- Manual measures on images to fit phyllotactic laws of development



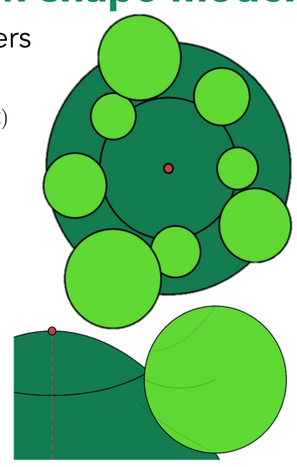
$r(\tau) = r_0 e^{\omega \tau}$



$Z(r) = Z_\rho + \zeta (r - \rho)^2$



$R(r) = R_0 e^{\alpha r}$



Deformable shape that defines a cylindrical referential and is indexed by (developmental) time

Parametric active model optimization: energy minimization by gradient descent in the parameter space

Optimize the shape model on the whole registered sequence point cloud

Energy functional with a classical formulation: **external** + **internal** terms

$$E(\mathcal{M}, \mathcal{P}) = \omega_{ext} E_{ext}(\mathcal{M}, \mathcal{P}) + \omega_{int} E_{int}(\mathcal{M})$$

Evaluated on a voxel grid

- External energy measures the fit to points: maximize the total density inside the model

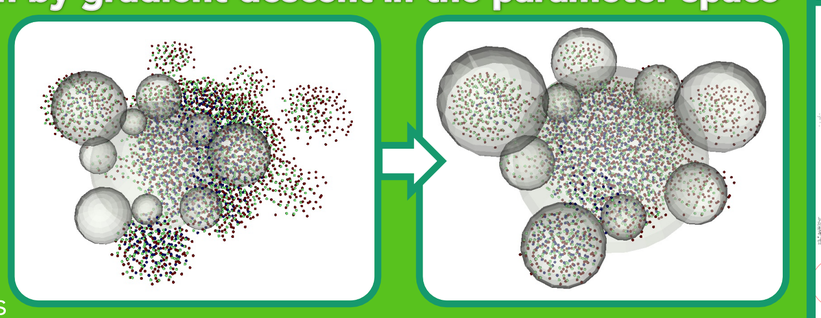
$$E_{ext}(\mathcal{M}, \mathcal{P}) = \sum_{\mathbf{x} \in \mathcal{M}} \left(\nu_{\min} - \nu_{\mathcal{P}}(\mathbf{x}) \right) \qquad \nu_{\mathcal{P}}(\mathbf{x}) = \sum_{P \in \mathcal{P}} \frac{1}{2} \left(1 - \tanh\left(\mathbf{k}(\|P - \mathbf{x}\| - \mathbf{R}) \right) \right)$$

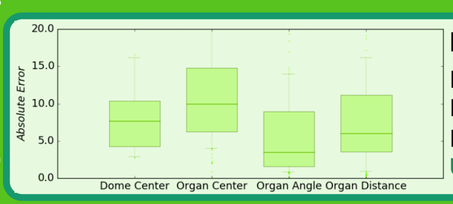
- Internal energy constrains the model shape: minimize the deviation from a vertical axis

Energy minimization by **iterative variation** of the model parameter values

- Compute the energy variation associated with moving each parameter in both directions
- Amplitude of variations and change probability controlled by a cycling temperature
- Orientation is set manually but could be optimized (separately from other parameters)

Possible **local optimization** of organ parameters afterwards (same energy)

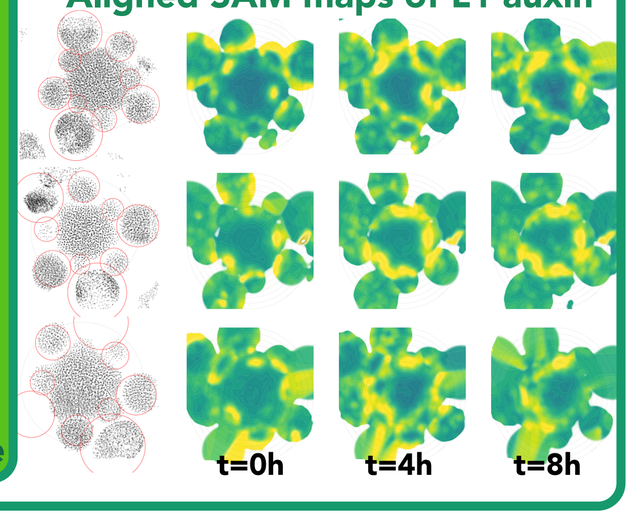




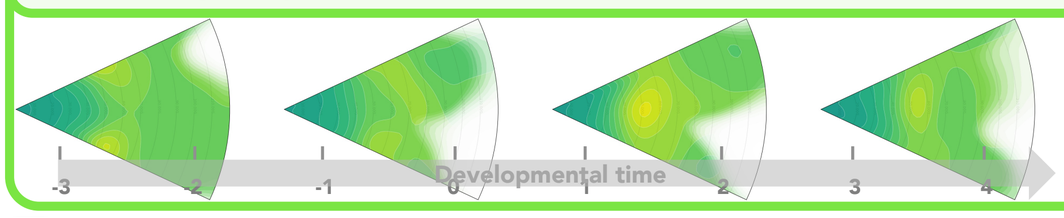
Evaluation of SAM model
Expertized meristem organs
Inexact young organ distance
High angular precision
Uncertain center positioning

Directly transform nuclei points into the 3D SAM referential & index the sequence by developmental time

Aligned SAM maps of L1 auxin



Average dynamics of auxin in primordium development: formation of an "inhibitory field" in the peripheral zone only



Formation of an auxin maximum from the center

- Constrained by older organs (depletion zone)

Appearance of a very focused auxin minimum

- Acting in the peripheral zone during 2-3 plastochrons

Directional inhibition (central ring) not isotropic

Perspectives of improvement & further developments

- Measure local spatio-temporal correlations of auxin & growth
- More accurate dome center location using CZ marker (**CLV3**)
- Better estimation of developmental time using auxin itself?

HUMAN FRONTIER SCIENCE PROGRAM
FUNDING FRONTIER RESEARCH INTO COMPLEX BIOLOGICAL SYSTEMS

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