Quantitative image analysis and identification of symmetry breaking events during pavement cell morphogenesis in Arabidopsis thaliana

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Introduction

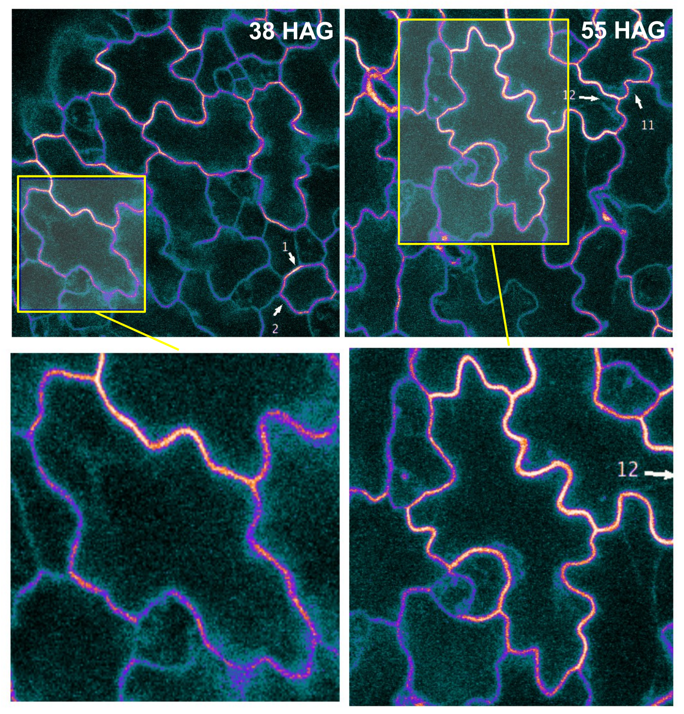

Figure 1. Example images of Arabidopsis thaliana cotyledon pavement cells at different time points after germination

Pavement cell growth in the cotyledon of Arabidopsis thaliana undergoes distinct phases that ultimately lead to the interlocking puzzle-piece morphology in the epidermis. First, cells undergo a patterning phase that leads to the emergence of lobes and this is subsequently followed by isotropic growth. To identify and quantify the process that govern the process of symmetry breaking and growth we developed a quantitative image-analysis strategy based on contracting convex hull applied to segmented cell shapes.

Method

The algorithm contains three parts: 1) convex-hull control point identification, 2) local minimum and inflection point tracking, and 3) a rule-based lobe point identification step. Parameter optimization of the algorithm results in a robust tool for large-scale identification of lobes, identification of lobing segments from multi-time point data, and provides a wealth of image-informatic descriptors for comparison testing.

❑ Lobe position estimation

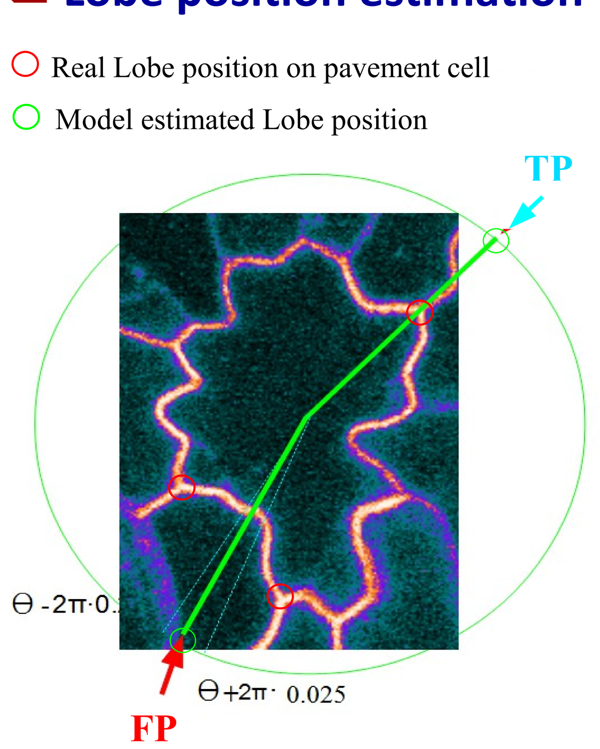

Figure 2. Lobe position estimation

	Lobe	Not Lobe
Positive	TP	FP
Negative	FN	TN

Positive result is defined when the estimated position(angle) is within the range between Θ (real lobe angle)$\pm 2\pi \cdot 0.025$. Negative result is defined outside the range. Four estimated conditions are True Positive(TP), False Positive(FP), False Negative(FN), and Ture Negative(TN).

Statistical measures of the estimate performance

Sensitivity = TP/(TP+FN)

FDR = FP/(TP+FP)

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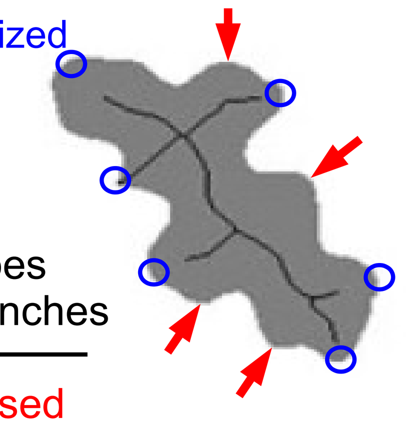
Problem

Previous methods based on skeletonized cells miss lobe initiation events and are ill-equipped to capture dynamic morphogenesis.



○ Lobe found by skeletonized cells method

➚ Lobe missed by skeletonized cells method

10 Lobes
6 Branches
4 Missed

Workflow

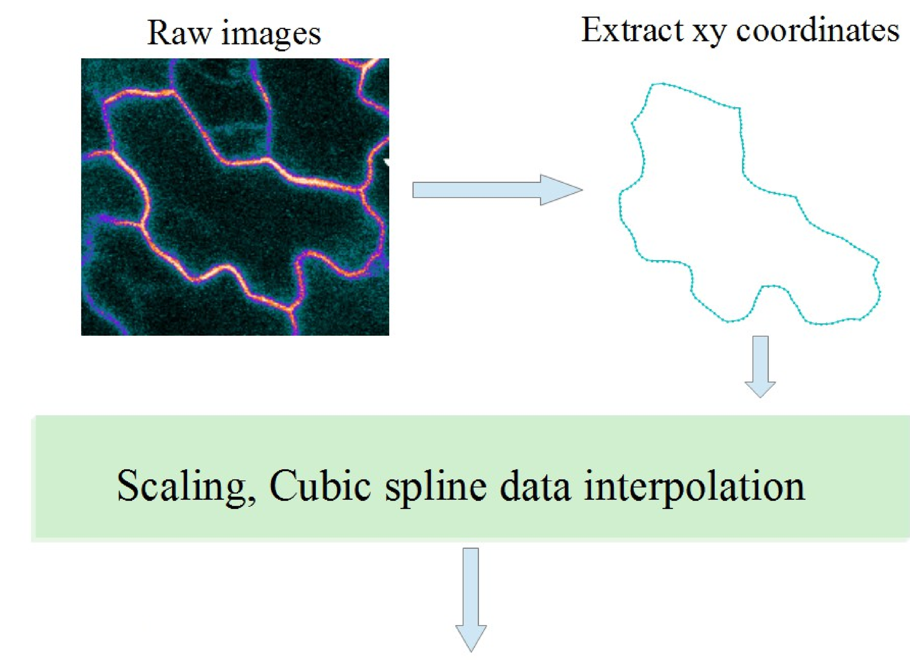
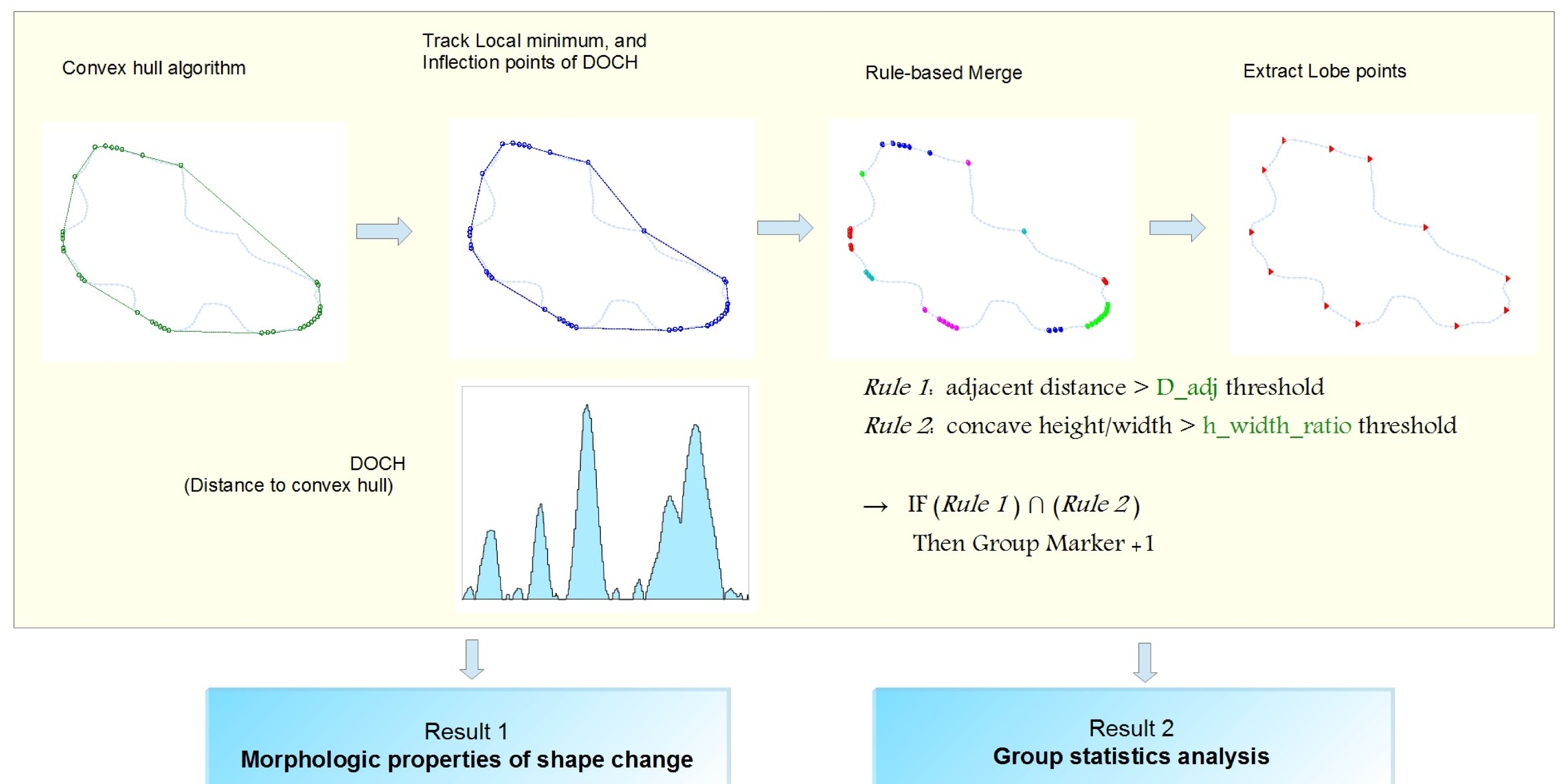
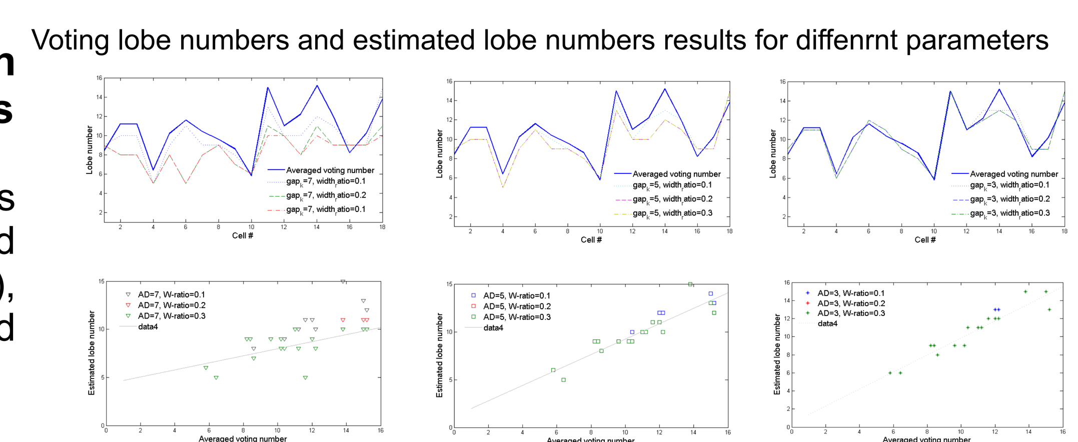



Figure 4. Workflow for the quantitative image-analysis strategy

Parameter optimization

Figure 5. Lobe number and position estimates results via different parameters

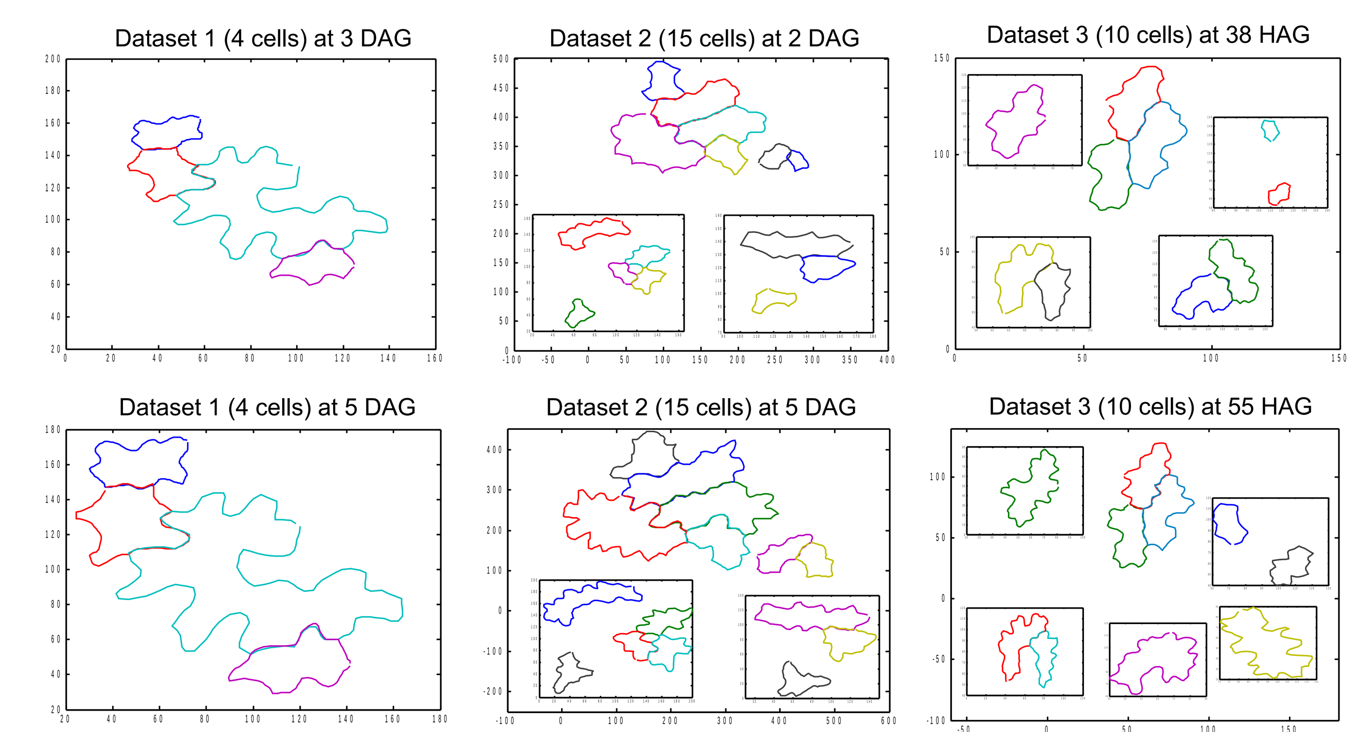


To determine the best model parameters including lobe adjacent threshold(gap_k) and concave height/width threshold (h_wd_ratio), different parameter set results were compared by lobe number and lobe location results.

Conclusions

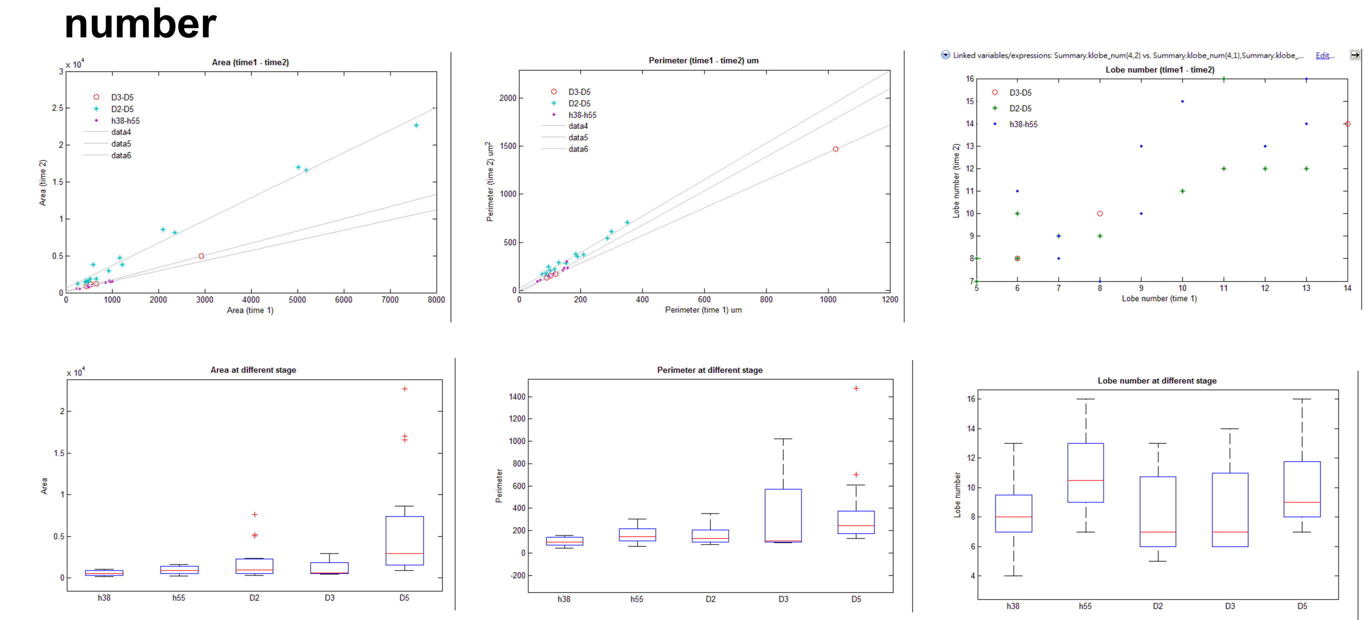
We developed a quantitative image-analysis convex-hull based strategy to study the dynamics process of pavement cell morphogenesis. High sensitivity(>0.95) and low False discovery rate(FDR) (<0.1) results show that the algorithm is robust for large-scale identification of lobes. Population statistics of the quantitative results demonstrate the distinct phases of lobe initiation events of developing pavement cells, and the ability to detect lobe initiation provides the necessary tools to unravel the mechanical and molecular mechanisms of pavement cell shape and cotyledon morphogenesis.

Results



❑ Population statistics of morphology properties

Figure 6. Population statistics of geometry features and lobe number



❑ Geometry index properties of pavement cell training set

Figure 7. Lobe number and lobe initiation events at different time stage datasets

	Dataset1 (N=4)		Dataset2 (N=15)		Dataset3 (N=10)	
	D3	D5	D2	D5	H38	H55
Averaged lobe number	8.7±3.6	9.0±3.6	7.9±0.1	9.9±2.4	9.3±2.3	11.6±2.3
%cell with lobe initiation	25		87		100	
Averaged new lobe initiation	1		2.0±1.56		2.30±1.10	
Junction number	6.75±1.71		6.67±1.05		7.00±1.41	

❑ ANOVA test of new initiation number with global properties

		Area	Convex Area	Perimeter	Convex Perimeter	Mean radius
new lobe initiation number	F value	1.532	1.647	1.854	1.349	1.372
	P	0.196	0.163	0.116	0.261	0.252

		Time	Compactness	Roundness	Convexity	Solidity
new lobe initiation number	F value	4.264	0.734	0.489	1.452	1.044
	P	0.002	0.626	0.812	0.222	0.414
		***				

F-Value = MSB/MSW

Figure 8. Results of ANOVA test of geometry feature, shape descriptor with lobe initiation events