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Support:

New Method for Epidermal Cells Detection in DIC images from *Arabidopsis thaliana* Leaves

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***Abstract*—*Arabidopsis thaliana* is the most widely used plant model for genetic analysis. Similarly, for cell cycle research, this model plant has been extensively used given the possibility of mutants phenotypic and molecular characterization in a straightforward manner. In this sense, the first pair of extended leaves and their cellular and morphological changes in a developmental context serve to understand the genetic control that regulates cell cycle progression. To characterize specific cell cycle mutants, their first pair of leaves are analyzed using a microscope attached to a tube to draw cells by hand. However, this process is tedious, inaccurate and highly inefficient. Although an imaging method for cells detection in *Arabidopsis thaliana* leaves was previously proposed, the technique did not always allow cell borders detection and therefore, manual editing was required. In order to make a more efficient and accurate analysis, the preliminary results were improved to develop a new technique for automatic cell detection, based on phase congruency, using DIC microscopy images. The obtained results after the application of this novel technique are here presented.**

***Index Terms*—DIC image , *Arabidopsis thaliana* , epidermal cells, phenotipic characterization, cell cycle regulation,phase congruency, cell detection, leaf**

I. Introduction

Arabidopsis thaliana is a useful model to understand the molecular events that regulate cell cycle control in plants [1]–[3]. Different researchers have shown the first pair of leaves of this plant is an optimal system to analyze the molecular events that regulate cell cycle progression. As the leaves grow, their cells progressively shift from an active phase of cell division to a phase in which leaf growth occurs by expansion of the cells. In the same way, the initiation of the cell expansion and the onset of differentiation process coincide with the molecular induction of an alternative cell cycle known as the endocycle. Therefore, gene alterations, environmental cues or stresses that directly influence the cell transition through the cell cycle, will have a direct effect on the phenotype of the plant. This phenotypic effect can be evidenced as a higher rate of cell division, increasing the final number of cells in the leaf or, on the contrary, can be characterized as an early induction of the differentiation process, whereby the increase in leaf area will be determined by cell expansion rather than by cell division and in this case, in contrast, the final cell number of the leaf will be smaller [1], [4]–[7].

In *Arabidopsis thaliana*, the phenotypic effect of a gene involved in the cell cycle process, can be accessed using a set of basic experiments, such as flow cytometric and count of epidermal cells [1]–[4], [8]. While the first process is fully automated and quantitative results can be obtained in minutes, the process of quantification and characterization of epidermal cells is highly laborious, tedious, error prone and temporarily inefficient because is based in the elaboration of serial drawings of different areas of the abaxial part in the first pair of true extended leaves after plant germination. Although a previous work for cell detection in *Arabidopsis thaliana* was published [9], the technique required manual editing, as the original images were quite contaminated by noise, as can be seen in Figure 1. Therefore, a new acquisition protocol was developed to obtain DIC images, which, as shown in Figure 6, allow to obtain better defined and continuous edges. However, since this is a new type of image, a new cell detection method is required. Therefore, a new image processing method for cell detection in *Arabidopsis thaliana* leaves is presented.

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Therefore, a new image processing method for cell detection in leaves of *Arabidopsis thaliana* is presented.

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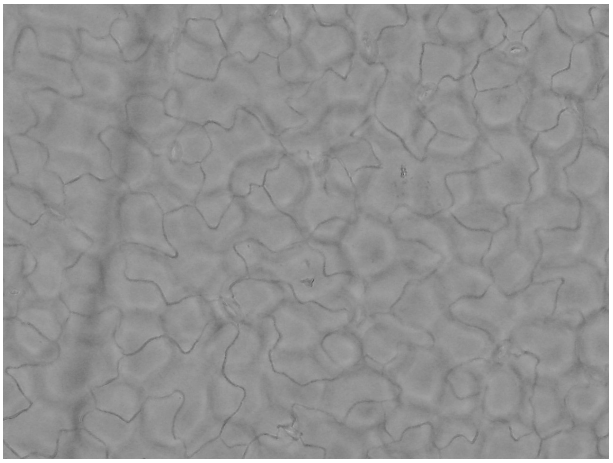


Fig. 1: Sample of microscopy *Arabidopsis* image.

II. Materials

For the acquisition of abaxial cell surface of epidermal tissue from *Arabidopsis thaliana*, plants with 21 days of post-germination growth (DAG) were used. Plants were processed by immersing the first real pair of leaves for 48 hours in acetone and transferred to a 1:9 acetic acid: 100% ethanol solution afterwards. Subsequently, cleared leaves were stored in lactic acid for microscopy. Epidermal cells were photographed under an optical microscope Carl Zeiss AXIO-Lab-A1 with a 10X magnification coupled to a Carl Zeiss-AxioCam ERc 5s camera. Forty images with a resolution of 1280 x 960 pixels were acquired and employed for testing. Ten of them were randomly chosen to validate the generated method for epidermal cells detection. The ground-truth of two images was traced by hand by a specialist. A sample image is illustrated in Figure 8.

The image processing algorithm was written in Java as a plugin of the free access software Imagej and developed on a computer Intel Core I7-353U processor (R) CPU 2.0GHz × 4 with 8 GB of RAM, running on Linux-Ubuntu 16.04 LTS operating system.

III. Methods

As shown in Figure 2, DIC images are characterized by edges that range from light to dark and from dark to light, and because cell walls are very thin, their profile is similar to the derivative of the impulse function, as can be seen in Figure 3. Thus, the cell wall border is located at the zero crossing of the function. However, the detection of zero crossings is not recommended, since any small variation in the background produces zero crossings that can be detected as false edges. Therefore, employing classical edge detection techniques, such as Prewitt and Sobel-Feldman gradient and Canny and Deriche edge detectors, multiple edges are produced on the resulting gradient magnitude image as shown in Figure 4 and Figure 5, where the magnitude profile, along the line marked in white in Figure 2, is shown. In contrast, phase congruence, a technique that generalizes edge detection, including ridges and valleys, is more appropriate in this case, as illustrated in Figure 6, and Figure 7, where the phase congruence profile, along the line marked in white in Figure 2, is shown. As can be seen, there is only a peak of great amplitude in the position where the cell wall is located.

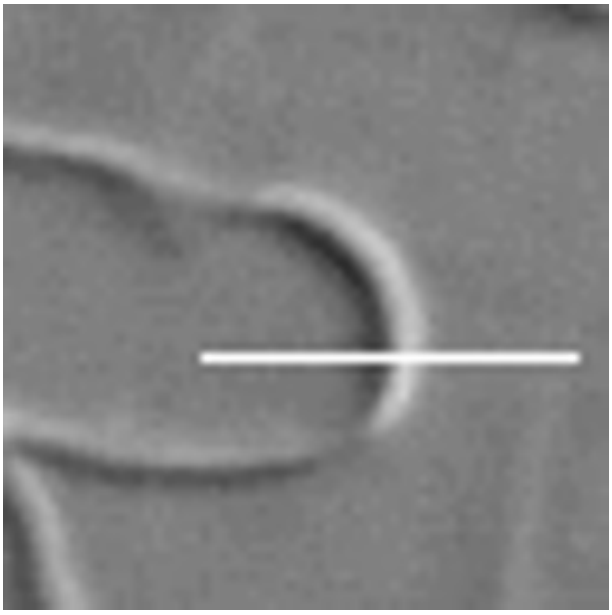


Fig. 2: Detail of a cell wall edge in a DIC image sample.

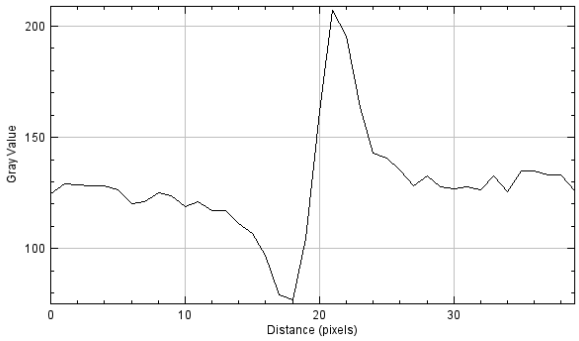


Fig. 3: Grey level profile along the line marked in white in Figure 2 .

The phase congruency, technique originally developed by Kovesi [10], allows the detection of edges in images by analyzing the phases of their frequency components. Since edges may be close to each other, parameters can be adjusted outside the range originally recommended by Kovesi to avoid overlapping of the LogGabor filter bank bands used to obtain adequate edges, as explained in [11].

Thus, the phase congruence was used to determine cell walls in DIC images, as the one illustrated in Figure 8, obtaining as a result an image, as shown in Figure 9, with values between 0 and 1, where zero means the non-phase coincidence and 1 the maximum congruence.

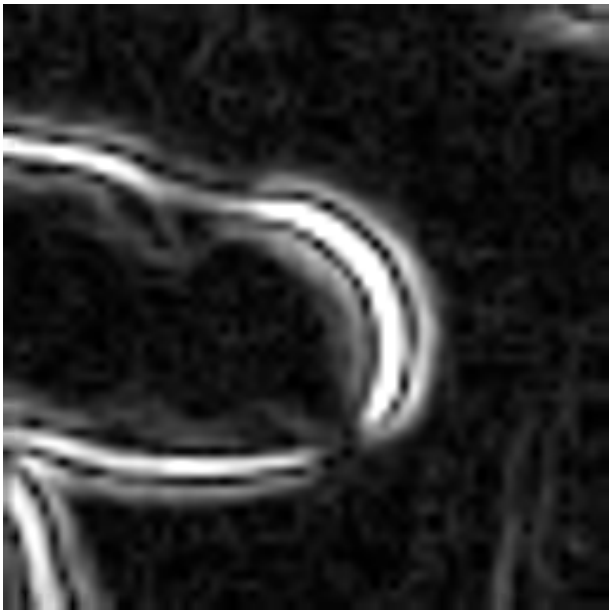


Fig. 4: Gradient magnitude of the image in Figure 2 obtained by using the Sobel-Feldman operator.

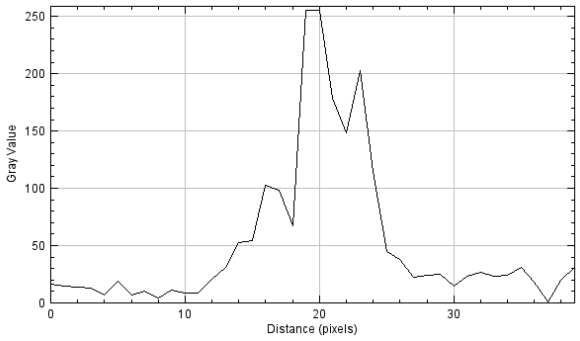


Fig. 5: Profile of the gradient magnitude, obtained by using the Sobel-Feldman operator, along the line marked in white in Figure 2.

Then, the PC image is binarized to separate the cell walls from the background. As shown in Figure 10, the typical histogram of the obtained phase congruence images is unimodal and has an exponential shape, so typical thresholding techniques are not suitable to separate the background from the object of interest. Thus, a threshold value $t = 100$ was chosen, which retains only 10% of the pixels with the highest PC value, assigning them as belonging to the cell walls. Figure 11 shows the threshold result obtained.

Once the threshold image is obtained, it is skeletonized to obtain the cell walls, as illustrated in Figure 12.

IV. Results

To evaluate the method, three of the *Arabidopsis thaliana* leaf images with different physical characteristics, belonging to the group of acquired samples, were taken. As can be seen in Figs. 13, 14, 15, the detection of the cell contours

Fig. 6: Phase congruence of the image in Figure 2.

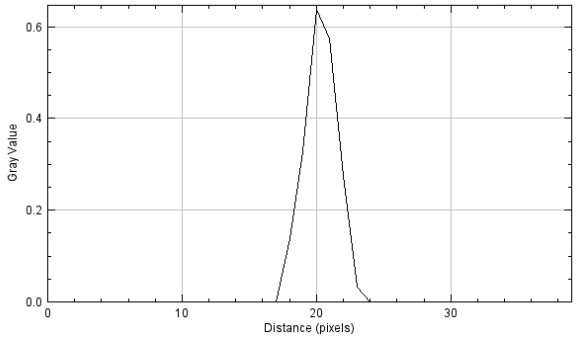


Fig. 7: Profile of the phase congruence, along the line marked in white in Figure 2.

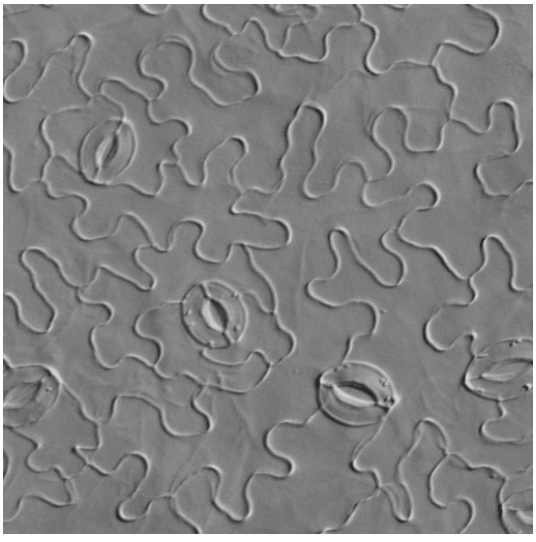


Fig. 8: DIC image of *Arabidopsis thaliana.*

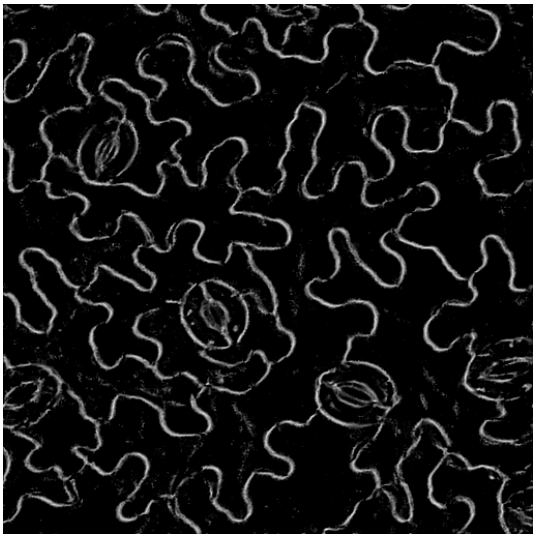


Fig. 9: Phase congruency of DIC image in (Figure 8.

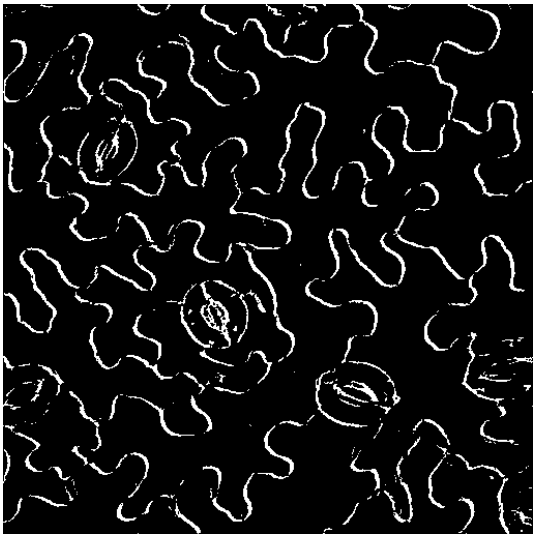


Fig. 11: Binarized image by using $t = 100$ as threshold.

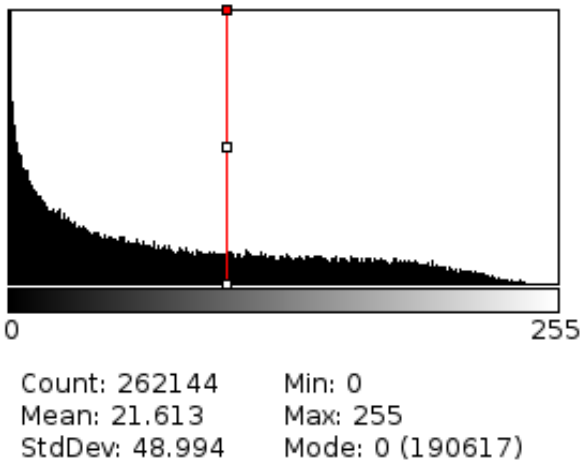


Fig. 10: Typical histogram of a DIC image of an *Arabidopsis thaliana* leaf. The threshold used to binarize the image is indicated in red.

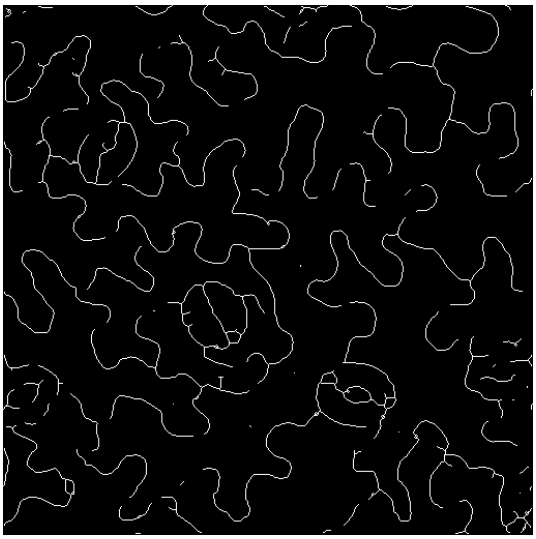


Fig. 12: Skeleton of image shown in Figure 12.

provided satisfactory results in all cases. Some cell walls present discontinuities, due to the fact that in several sections they present very little contrast. Thus, to achieve the best results it is necessary to establish the best possible acquisition protocol to produce the best possible result.

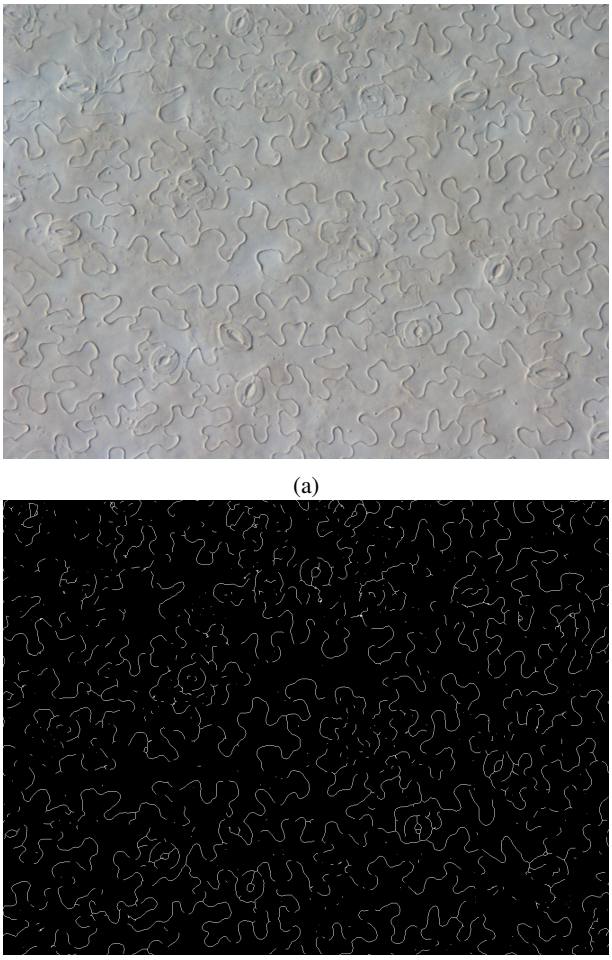
The average time needed by the program to detect epidermal cells in the three test images was 13.202 ± 0.103 seconds, requiring only a small post-editing to correct the results. This time is less than that required by the method previously developed by Forero et al., evaluated on the same DIC images, before editing, which was 16.583 ± 0.148 seconds and much lower than the time required by the manual process.

The Dice index was used to evaluate the proposed method. Since the technique previously developed by Forero et al. [9] was not implemented for this kind of images, the results as observed in Figure 16 produce double edges, in the vicinity of the cell wall, and, therefore, the results of both techniques cannot be compared, using the Dice index, since the intersection between the result obtained with the previous technique and the ground truth image will be empty, and, thus, the Dice index will be equal to zero. Table I summarizes the Dice score obtained for each image. The resulting images are illustrated in Figs. 13b, 14b and 15b.

TABLE I: Results Dice Score

Image	Dice Score
A	0.8429
B	0.8736
C	0.8367
Average	0.8511

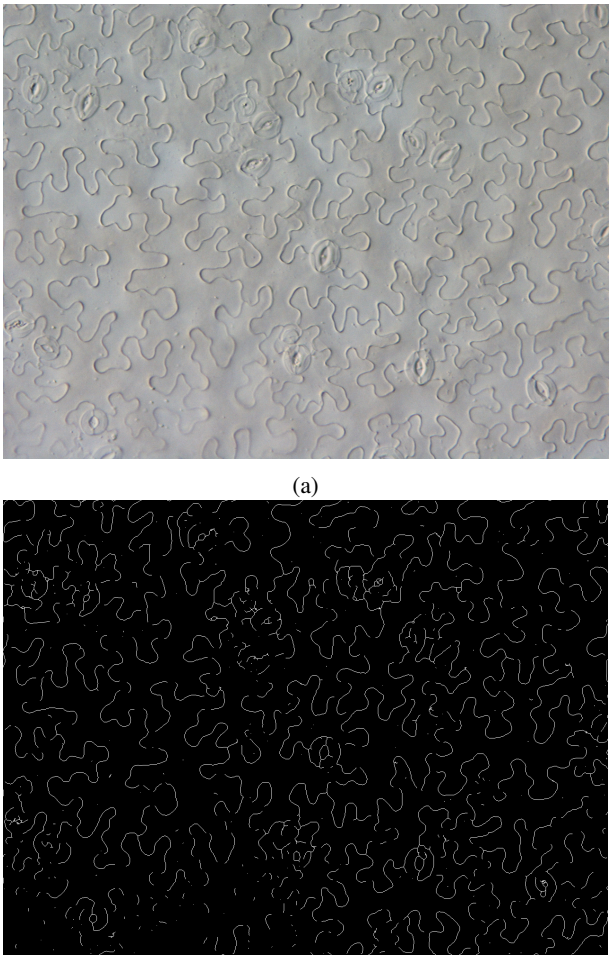
The Dice score obtained is greater than the average score obtained by the Forero 2019image method, 0.8325, which is



(a)

(b)

Fig. 13: Sample A. (a) DIC image. (b) Result image.



(a)

(b)

Fig. 14: Sample B. (a) DIC image. (b) Result image.

given by the number of pixels at the intersection between the resulting image and ground truth divided by the total number of pixels in the second one, i.e., this measure is less drastic than the Dice score and therefore the Dice score of the previous technique is even less than 0.8325.

V. Conclusions

To study the cell cycle in *Arabidopsis thaliana*, the characterization of the epidermal cells in size and number is one of the most important contributions that must be considered to describe a specific phenomenon. Therefore, this description implies that all cells must be detected.

In this work, a new technique was introduced for the detection of epidermal cells in *Arabidpsis thaliana* leaves from DIC images, which has as advantage a better edge definition and less noise than those obtained by bright field microscopy. However, DIC images are characterized by shadow-casting appearance, i.e. the edges are not located on the maximum or minimum intensity pixels. Therefore, traditional edge detection techniques do not yield good results. Therefore, traditional edge detection techniques do not yield good results.

In this work, we introduce the phase congruence to detect cell walls, obtaining a good detection and edges, with a score of 0.8511 on average, higher than that obtained by a technique used on bright field microscopy images. These preliminary results show the capacity of this technique and can be improved with additional post-treatment.

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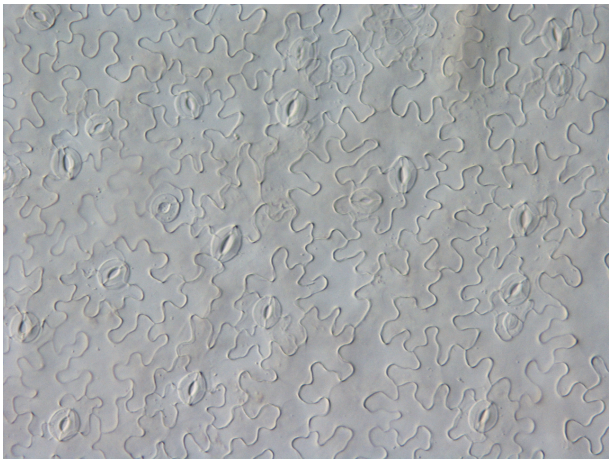
References

[1] G. T. Beemster, L. De Veylder, S. Vercruysse, G. West, D. Rombaut, P. Van Hummelen, A. Galichet, W. Gruissem, D. Inzé, and M. Vuylsteke,



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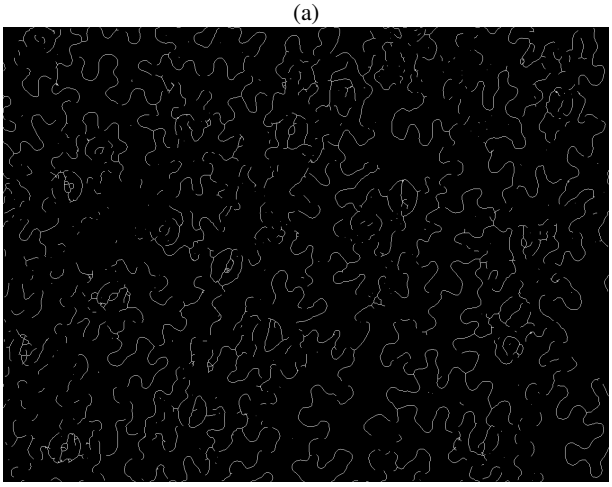

(b)

Fig. 15: Sample C. (a) DIC image. (b) Result image.

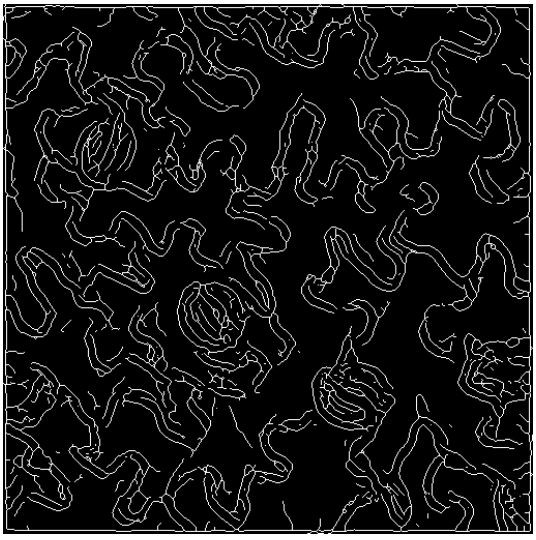

Fig. 16: Resulting image obtained by applying the method of Forero et al. [9] on the DIC image shown in Figure 8 . It should be noted that this technique was not developed to be used in this kind of images, but allows to compare the execution time of both algorithms.

"Genome-wide analysis of gene expression profiles associated with cell cycle transitions in growing organs of arabidopsis," *Plant physiology*, vol. 138, no. 2, pp. 734–743, 2005.

[2] L. De Veylder, T. Beeckman, G. T. Beemster, J. de Almeida Engler, S. Ormenese, S. Maes, M. Naudts, E. Van Der Schueren, A. Jacqmard, G. Engler *et al.*, "Control of proliferation, endoreduplication and differentiation by the arabidopsis e2fa–dpa transcription factor," *The EMBO journal*, vol. 21, no. 6, pp. 1360–1368, 2002.

[3] K. Vlieghe, V. Boudolf, G. T. Beemster, S. Maes, Z. Magyar, A. Atanassova, J. de Almeida Engler, R. De Groodt, D. Inzé, and L. De Veylder, "The dp-e2f-like gene del1 controls the endocycle in arabidopsis thaliana," *Current Biology*, vol. 15, no. 1, pp. 59–63, 2005.

[4] V. Boudolf, K. Vlieghe, G. T. Beemster, Z. Magyar, J. A. T. Acosta, S. Maes, E. Van Der Schueren, D. Inzé, and L. De Veylder, "The plant-specific cyclin-dependent kinase cdkb1; 1 and transcription factor e2fa-dpa control the balance of mitotically dividing and endoreduplicating cells in arabidopsis," *The Plant Cell*, vol. 16, no. 10, pp. 2683–2692, 2004.

[5] J. de Almeida Engler, L. De Veylder, R. De Groodt, S. Rombauts, V. Boudolf, B. De Meyer, A. Hemerly, P. Ferreira, T. Beeckman, M. Karimi *et al.*, "Systematic analysis of cell-cycle gene expression during arabidopsis development," *The Plant Journal*, vol. 59, no. 4, pp. 645–660, 2009.

[6] Z. Magyar, B. Horvath, S. Khan, B. Mohammed, R. Henriques, L. De Veylder, L. Bakó, B. Scheres, and L. Bögre, "Arabidopsis e2fa stimulates proliferation and endocycle separately through rbr-bound and rbr-free complexes," *The EMBO journal*, vol. 31, no. 6, pp. 1480–1493, 2012.

[7] D. Yi, C. L. A. Kamei, T. Cools, S. Vanderauwera, N. Takahashi, Y. Okushima, T. Eekhout, K. O. Yoshiyama, J. Larkin, H. Van den Daele *et al.*, "The arabidopsis siamese-related cyclin-dependent kinase inhibitors smr5 and smr7 regulate the dna damage checkpoint in response to reactive oxygen species," *The Plant Cell*, vol. 26, no. 1, pp. 296–309, 2014.

[8] Sternberg, "Biomedical image processing," *Computer*, vol. 16, no. 1, pp. 22–34, 1983.

[9] M. G. Forero, S. A. Perdomo, M. A. Quimbaya, and G. F. Perez, "Image processing method for epidermal cells detection and measurement in arabidopsis thaliana leaves," in *Iberian Conference on Pattern Recognition and Image Analysis*. Springer, 2019, pp. 416–428.

[10] P. Kovesi, "Image features from phase congruency," *Videre: Journal of computer vision research*, vol. 1, no. 3, pp. 1–26, 1999.

[11] C. A. Jacanamejoy and M. G. Forero, "A note on the phase congruence method in image analysis," in *Progress in Pattern Recognition, Image Analysis, Computer Vision, and Applications*, R. Vera-Rodriguez, J. Fierrez, and A. Morales, Eds. Cham: Springer International Publishing, 2019, pp. 384–391.