Identification and Automatic Counting of Stomata through Epidermis Microscopic Images of a leaf

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Abstract— Stomata are the small pores on a surface of leaves which are responsible for the process of respiration. These stomata help plants for intake of carbon-dioxide and release of oxygen. This paper proposes method of identification and automatic counting of stomata on the microscopic image of an epidermis of a leaf. There are various methods of detection and counting of stomata. This paper uses the method of detection and automatic counting of stomata by using morphological operations. There are various morphological operations used in this paper which include dilation, erosion and opening by reconstruction, closing by reconstruction etc. This is most efficient method than others because it gives the efficiency of 94%.

Key words: Stomata; Epidermis; Morphological operation; Opening by reconstruction; closing by reconstruction

I. INTRODUCTION

Stomata are a pore on epidermis of plant leaf that allows an exchange of gases between the leaf tissue and atmosphere [1]. Photosynthesis is an important process of plants in which intake of carbon-dioxide and release of oxygen take place. This process converts the Sunlight energy into usable energy for plant growth. Plant regulates thermal cooling due to water loss allowed by stomata opening. This is controlled and triggered process by the stomata opening and closing. Each cell is surrounded by a specialized cell called as guard cells. These guard cells enable stomata open or closed which causes transpiration. The number of stomata cells on a leaves changes with respect to factors in environments like light and temperature, found in the areas where the plants growth takes place [2], [3].

Stomatal characteristics also explain much of intraspecific variability of regulating the transpiration under deficiency of water in plants [1].The stomatal density in high light area is more than that in low light area. The stomatal density is primary feature because it can affect the diffusive resistance of stomata. Thus the increase in stomatal density is an important factor for the decreasing of stomatal diffusive resistance [7].

Understanding the morphology and structure of stomata and modelling their variation across different species under different environmental conditions are important to plant biologist in order to understand plant development [6]. To accomplish this aim practically, plant biologist take microscopic images of a plant leaves and manually count the number stomata present per unit area on the leaves, evaluate opening of each stomata and size, shape of their guard cells [6].

This procedure performs again and again over number of images of different species of plants. Analysing numbers of stomata per unit area is a vital task for analysis of plants. However tools available for identification of stomata are limited [4]. Due to this reason, the goal of this study is to design software for automatic counting and detection of stomata from microscopic images of epidermis of plant leaves.

The first method is detection and counting of stomata by using morphological operation and the second is to design tool for identification and counting of stomata by using template matching algorithm. There are various methods of identification and counting of stomata in robotics communities and computer vision, e.g. template matching Algorithm [5], statistical models [6], can be employed.

This paper proposes the method of detection and automatic counting of stomata by using morphological operation. This method uses number of processes on the images which starts from the gaussian filter then performs morphological operations of image processing. The results of stomata detection and counting by this method showed an accuracy of 94% of the stomata of 24 images from 5 plant species [7].

II. LITERATURE SURVEY

Plant biologist use software named as image J Software to count and segment stomata cells from leaf images and to study the properties of stomata.While other technique can gives good response in case of accuracy. They are slow and complex and cannot used where hundreds of images need to be processed in a single step.

R. C. Gonzales and R. Woods described various morphological operations which are used for automatic identification and counting of stomata cells 6]. This technique uses various morphological operations which starts from Gaussian low pass filter and number of processes are performed which includes various morphological operation.

Karabourouriotis et al. [10] uses fluorescent microscope takes the images of a plant leaves and count number stomata on the leaves epidermis. This process use Illumination Correction Filter (ICF) to processed captured images in order to correct illumination. Then median filter is used to filter the images and thresholding is done to remove background. The objects which remain are nothing but the stomata cells. There is one assumption that background operated on fluorescent images and relatively uniform.

Sanyal et al. [8] uses image processing techniques to measure the morphological features of stomata cell of leaves of tomato. This approach aims n images that contain single stomata without any additional features in the background. This makes segmentation and detection process disputing.

Jones et al. [9] reviewed and discussed strategies which is used in the technique of thermal imaging for knowledge of conductance of stomata in the field and compare techniques of collection of image and analysis.

This technique reviewed concentrate on measuring temperature of leaf and distribution of canopy temperature which gives nothing but the indication of opening of stomata, although these techniques are unable to give direct measurement of stomatal morphological features.

Finally, we reviewed that there are many standard object recognition techniques which are popular among the robotics communities and computer vision. These techniques are template matching [10], statistical models [6] [7], Background subtraction technique [`13] or active shape models [14] can be used in future.

III. METHODOLOGY

In response to identify the stomata in microscopic images of leaf, we have implemented the technique of mathematical morphology. The mathematical morphology is a technique and theoretical model for the processing of geometrical structures and analysis based on, lattice theory, random functions, topology and set theory [5]. The basic concept of image processing by using mathematical morphology is nothing but the use of some

Morphological operators that is strongly concerned to concept of Minkowski addition. The development of the mathematical morphology was originally done for binary images, and was then advanced to grayscale images. In time and frequency domains, the main and important morphological operators are dilation, erosion, closing and opening [6]. In morphology of grayscale the dilation of by b, denoted f ⊕ b, is given by

$$(f \oplus b)(x) = sup_{y \in E} \; [f(y) + b(x-y)], \qquad (1)$$

Whereas the structuring function is represented by b(x), the original image given by f(x), and E is the domain of f. Similar to the case of dilation, the erosion of f by b, represented as f ⊖ b, is given as follows.

$$(f \ominus b)(x) = inf_{y \in E} \; [f(y) - b(y-x)], \qquad (2)$$

Just like in morphology for binary images, the opening and closing operations are given respectively by

$$(f \circ b) = (f \ominus b) \oplus b \quad \text{and} \qquad (3)$$

$$(f \bullet b) = (f \oplus b) \ominus b \, , \qquad (4)$$

This paper proposes two main morphological operations which are opening by reconstruction and closing by reconstruction described below sequentially.

A. Opening-By-Reconstruction

In an opening of mathematical morphology, the main purpose of erosion is to remove small objects and the main function of dilation is to restore the remaining shape of objects after erosion. On the other hand, this restoration's accuracy morphological is heavily dependent on the structuring element used and similarity of the shapes of the objects The main purpose of opening-by reconstruction is to restore the remaining shapes of the objects after erosion [5].

The opening-by-reconstruction of the image f by a structuring element b, consist of an opening of f by b, then the reconstruction from the opened image. This operation is represented by [5]:

$$r_f(f \oplus b) \qquad (5)$$

The reconstruction of morphology is a transform which includes a structuring element and two images [7]. Out of these two images, first image is marker which consists of the starting elements for the purpose of transformation. The second image, the mask limits the transformation. The connectivity between the pixels is defined by the structuring element. This process can be called as repeated dilations of marker image, till the margin of the marker image fits under the other image, called as mask image. In reconstruction of morphology, the peaks values in the marker image dilate or spread out.

B. Closing-By-Reconstruction

The main purpose of closing operation is to fill in the small gaps in image and smoothing outer edges of the image. On the other hand, the closing-by reconstruction, try to conserve the original edges of the shapes [7].

Closing by reconstruction is dual of the opening-by-reconstruction, defined as a closing of by the structuring element b followed by a morphological reconstruction can be denoted by [5]:

$$r_f(f \ominus b) \qquad (6)$$

C. Detection of Stomata Cell by Morphological Method

At a first look at the original epidermis microscopic image as shown in figure 2a; we have seen that all stomata circular in shape along with two visible regions namely center and border. These features can be used when we were trying to detect the stomata cells between all other images of leaf structures. To implement this thought, the foreground and background markers are computed. These markers are defined as connected blots of pixels within each of the background or the objects. In order to do that we can use the above cited operators to find the stomata in leaf epidermis images. The steps explained below sum up the complete process.

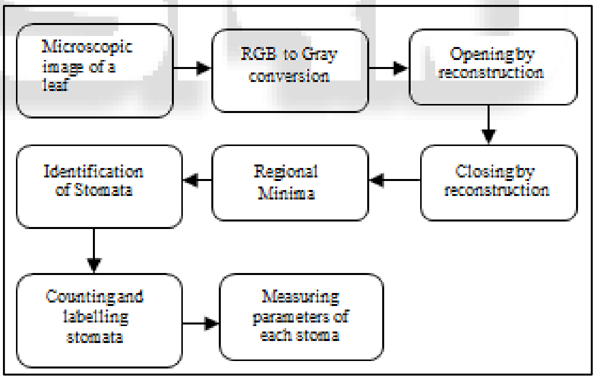


Fig. 1: Block Diagram Stomata Detection and counting

In order to eliminate some noise, we apply a Gaussian low-pass filter on the image. We utilize a filter with parameter σ = 0.5 and size 25 × 25 pixels. The results of this process can be seen in Figure 2b. After that, morphological operation named as opening by reconstruction is performed over the image.

As discussed before, the opening is defined as erosion performed after the dilation; on the other hand the opening -by-reconstruction is defined as erosion performed after a morphological reconstruction. This process helps to retain the original shapes of the particular objects that stay after the process of erosion. The objective here is to flatten the objects of interest, removing grayscale variation inside stomata cells. Figure 1c shows the result of this process. We can note that stomata and the background are now flattened with respect to their gray-scale value.

The next step is to perform a closing-by-reconstruction in order to fill the remaining gaps in the flattening regions (a kind of 3 noise removal). This morphological technique will remove the noise present in the resultant image. These operations will produce flat minima inside each object; the result of this step has been shown in figure 2d.

With this image without noise, the next step is to identify what is background and what are the stomata. To achieve that we calculate the regional minima of I. Regional minima is defined as connected pixel's components with a fixed value of intensity, and whose all the pixels of external boundary have a higher value. As we can see in Figure 2d, after performing the morphological process, if we compared the stomata region with their neighbourhood present low values. Figure 2e shows the result of this procedure.

IV. Result and Discussion

On the resultant image we have seen that, stomata and some other structures of leaf were identified. To eliminate the spurious identifications we have used a simple rule where the objects having a perimeter twice larger than the mean perimeter of all objects of the microscopic image of leaf are removed of the selection. The final result is the image with all stomata identified in Figure 1f.

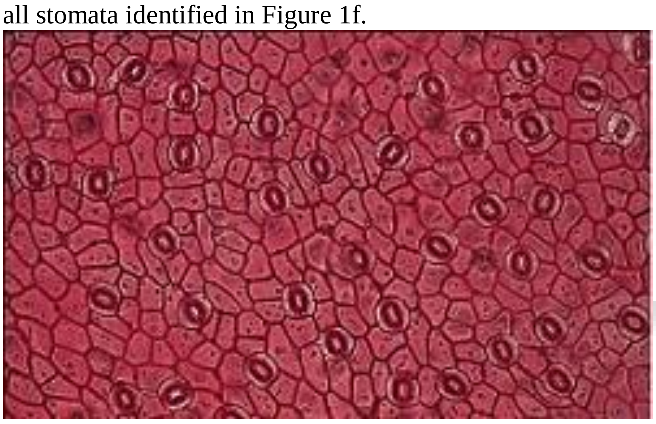


Fig. 2: Original image

Above image shows original microscopic image of a leaf. This image is in a RGB form, as morphological operation will work only on binary or gray scale images. Thus we need to convert it into gray scale image as shown in fig.2 b)

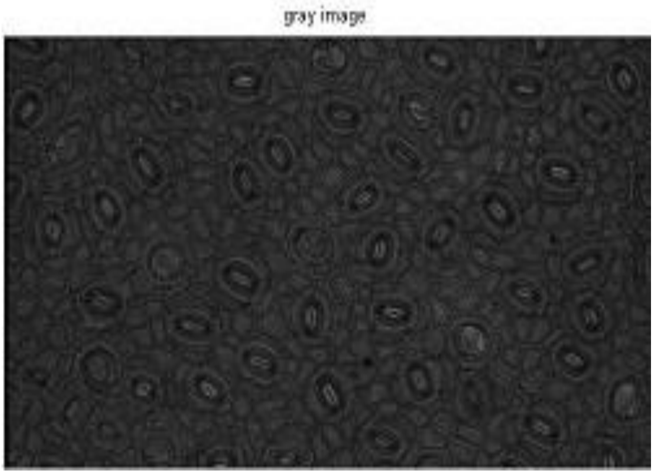


Fig. 3: Gray scale image

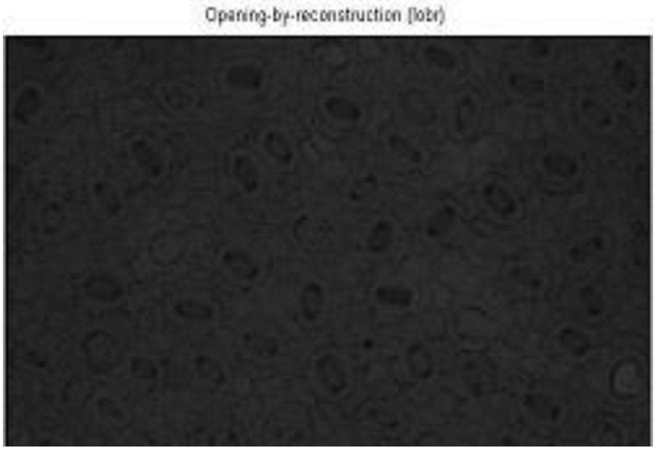


Fig. 4: Opening by Reconstruction

In fig.2c) shows the image after performing opening by reconstruction operation. We can see that the object of interest is flattened and gray scale variation inside each stoma is removed.

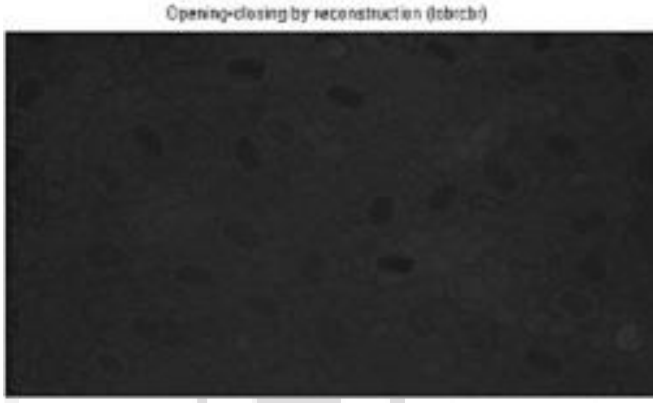


Fig. 5: Opening-Closing by Reconstruction

Above fig. 2d) shows an image after performing opening-closing by reconstruction. This operation fills the small gaps in the flattening region. It also provides smoothness to outer edges and attempt to preserve the original edges of an object
of interest.

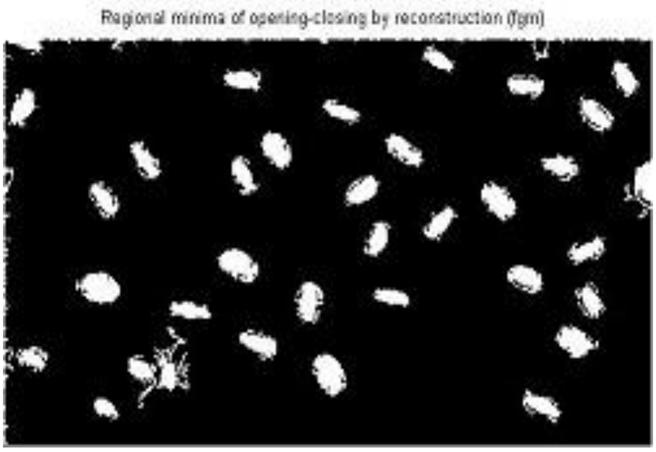


Fig. 6: Regional minima of an original image

Above fig. 2e) determines the results of performing regional minima operation. This operation determines stomata and background separately. This is because of all stomata has constant intensity value.

Fig. 7: Clear edges after erosion

Above fig.2f) shows an image after performing erosion operation. This image also shows the clear edges. This operation also removes the noise present in an image.

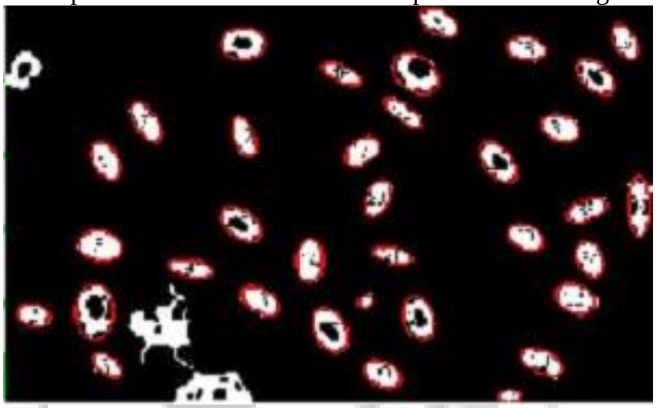
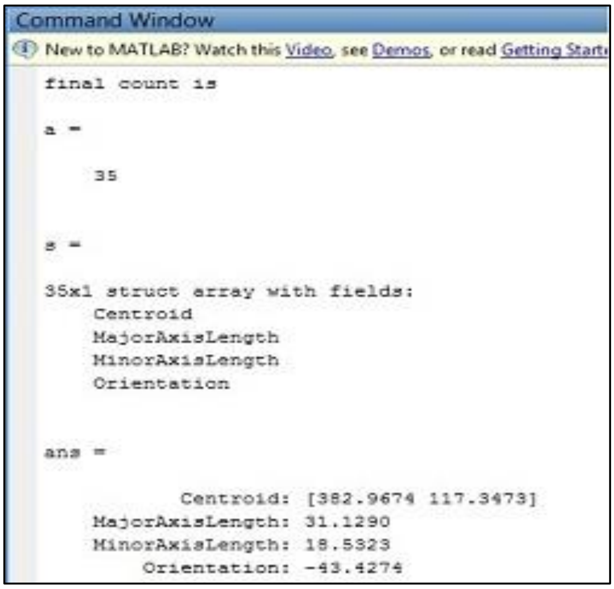


Fig. 8: Resultant image showing labeling and counting of stomata

Above fig.2g) shows the resultant image which shows the counting of stomata and each stoma has labelled its number of count. These stomata are aligned in different direction. The curve made by red line shows the curve for orientation of stomata.



```
Command Window
New to MATLAB? Watch this Video, see Demos, or read Getting Start

final count is

a =

    35


s =

35x1 struct array with fields:
    Centroid
    MajorAxisLength
    MinorAxisLength
    Orientation


ans =

           Centroid: [382.9674 117.3473]
    MajorAxisLength: 31.1290
    MinorAxisLength: 18.5323
        Orientation: -43.4274
```

Fig. 9: Command window showing feature of stomata

Fig.3 shown above indicates the morphological features of individual stoma. These morphological features are length, breadth, centroid and orientation of stomata. These values are measured in the form of pixel value as it is directly converted into micrometer value.

V. CONCLUSION

In a result it is shown that although the problem of identification of stomata is complex and very important for the analysis of plants, there is still deficiency in material aiding this issue. However, the proposed approach showed that with simple computational tools is possible to obtain good results. This fact was demonstrated by the high rate of identification of stomata in epidermis microscopic images. These promising results leave open a range of possibilities for future works, among which the use of other computational tools for segmentation of stomata and their application in the field of botany.

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REFERENCES

[1] R. C. Gonzales and R. Woods, Digital Image Processing. Addison, Wesley, 1992

[2] G. Karabourniotis, D. Tzobanoglou, D. Nikolopoulos, and G. Liakopoulos, “Epicuticular phenolics over guard cells: exploitation for in situ stomatal counting by fluorescence microscopy and combined image analysis,” Annals of Botany, vol. 87, no. 5, pp. 631–639, 2001.

[3] P. Sanyal, U. Bhattacharya, and S. Bandyopadhyay, “Analysis of sem images of stomata of different tomato cultivars based on morphological features,” in Modeling Simulation, 2008. AICMS 08. Second Asia International Conference on, May 2008, pp. 890–894.

[4] H. G. Jones, M. Stoll, T. Santos, C. De Sousa, M. M. Chaves, and O. M.Grant, “Use of infrared thermography for monitoring stomatal closure in the field: application to grapevine,” Journal of Experimental Botany,vol. 53, no. 378, pp. 2249–2260, 2002.

[5] H. Giday, K. H. Kjaer, D. Fanourakis, and C.-O. Ottosen, “Smaller Stomata require less severe leaf drying to close: A case study in Rosa Hydrida,” Journal of Plant Physiology, vol. 170, no. 15, pp. 1309 – 1316, 2013.

[6] Hamid Laga, Fahimeh Shahinnia, Delphine Fleury, “Image- Based Plant Stomata Phenotyping”, 2014 13th International Conference on Control, Automation, Robotics & Vision Marina Bay Sands, Singapore, 10-12th December 2014 (ICARCV 2014)

[7] R. Pandey, P. M. Chacko, M. Choudhary, K. Prasad, and M. Pal, "Higher than optimum temperature under {CO2} enrichment Scientia Horticulturae, vol.113, no. 1, pp. 74 – 81, 2007.
[8] C. A. Sweeney, "A key for the identification of stomata of the Native conifers of scandinavia," Review of Palaeobotany and Palynology, vol.128, no. 3–4, pp. 281 – 290, 2004.