

A Review: Methods of Automatic Stomata Detection and Counting Through Microscopic Images of a Leaf

Nitin Bhaiswar¹, Dr.V. V. Dixit²P.G. Student, Department of E&TC, Sinhgad College of Engineering, Vadgaon (BK), Maharashtra, India¹Professor, Department of E&TC, Sinhgad College of Engineering, Vadgaon (BK), Maharashtra, India²

ABSTRACT: Stomata are the small pores in leaf epidermis of a plant which are important for the intake of carbon-dioxide and release of oxygen for the growth of plant. Our aim is to discuss methods to design a tool which can automatically detect number of stomata present on an epidermis of a leaf and count them. First method is by using morphological operation and another by using the template matching algorithm. These methods also measure their stomatal and morphological features.

KEYWORDS: Epidermis, Morphological operation, Template matching algorithm

I. INTRODUCTION

Stomata are a pore on plant leaf that allows 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 specialised cell called as guard cells. These guard cells enables stomata open or closed which causes transpiration. The number of stomata cells on a leaves changes with respect to factors in environment 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 number of stomata per unit area is an important factor for the decreasing of diffusive resistance of stomata [7].

Understanding the morphology and structure of stomata and modelling their variation across various species under changing environmental

Conditions are necessary for the 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.

This paper proposes two methods for identification and counting of stomata which will be discussed sequentially. 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.

The first 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 94.3% of the stomata of 24 images from 5 plant species [7].

The second method design a software to automatically identify and count stomata features of the images. The main challenge in developing such a tool is the lack of contrast between stomata cells and the surrounding background [6].

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This method utilises template matching algorithm to find stomata one by one and analyses to count features of stomata within the regions of stomata. This process starts from the building a database of n templates which we then segment from few images.

We then improve the database by rotating and scaling original template which give rise to database of n templates ($N \geq n$) [6]. This help us to detect cells of stomata at different orientation and scales when given image is convolved with an each template image in the database and enter the maximum result at every pixel into the map M .

The locations of stomata are then detected as local maxima of the map M . There are several advantages of this method. First is with detection of stomata we get the original shape of each stomata cells. Other it enable us to manage the vast variation in the stomata appearance and shape.

II. RELATED WORK

Plant biologist use software named as image JSoftware to count and segment stomata cells from leaf images and to study the properties of stomata. While other technique can give 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 [10] uses fluorescent microscope take 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 object which remains is nothing but the stomata cells. There is one assumption that background operated on fluorescent images and relatively uniform.

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

Jones [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. This technique is 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. METHODS OF AUTOMATIC STOMATA DETECTION AND COUNTING

1. Method of stomata detection and counting by template matching method

This method uses various images as a database in the form of templates of leaf images. Then the features of leaf image are matched with stored images in a database. This process helps to design a tool which automatically detect and count Stomata cells on the epidermis of a leaf image

A. Image acquisition:

We consider the wheat plant's leaf which is at the stage of anthesis. We put a chemical adhesive named as Cyanoacrylate (Supaglu) on the leaf surface of a plant and slowly place it between slides of microscope. We split the slides and the leaf is removed. This will gives an impression of leaf. This impression of a leaf becomes useful for the capturing various image of a leaf under the microscope [6].

B. Detection of stomata:

One of the main difficulties in performing processes on the various types of images is that the stomata cannot be easily detectable from their surroundings: the colour contrast of those images is very low and in case of size and shape they

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share many resemblances with other parts of leaves... As a consequence, detection by using the method of colour thresholding or primitive fitting would require setting various parameters that are dependent on images. This method proposes an efficient way of stomata detection by using template matching.

Preprocessing: The purpose of Preprocessing is to change the input image into a format which is robust to changes in process of acquisition. This is done by withdrawing features which are slow in variation in the image and increasing the area of regions of interest. Suppose I be the input image in grayscale format. Our first process is called normalization, which subtracts the mean from the image I ($I \leftarrow I - \frac{1}{n} \sum I(i, j)$, where n represents the total number of pixels), and gaussian filter $G\sigma$ used to smoothen the resultant image of zero mean and $\sigma = 3$, ($J \leftarrow G\sigma * I$). Finally, by subtracting J from I ,

Slow varying features are removed. Binarizing the result and performing histogram equalization by tuning all the pixels which are having a value higher than 0.5 to 1 and the others to 0. This will result in a binary image that describes the walls of stomata see Figure 1(b) for an example. However, this process also describes other parts that are related to various features in the leaves of plants. Since we concentrate on features of stomata only, an additional processing is required which is called as stomata morphology [6].

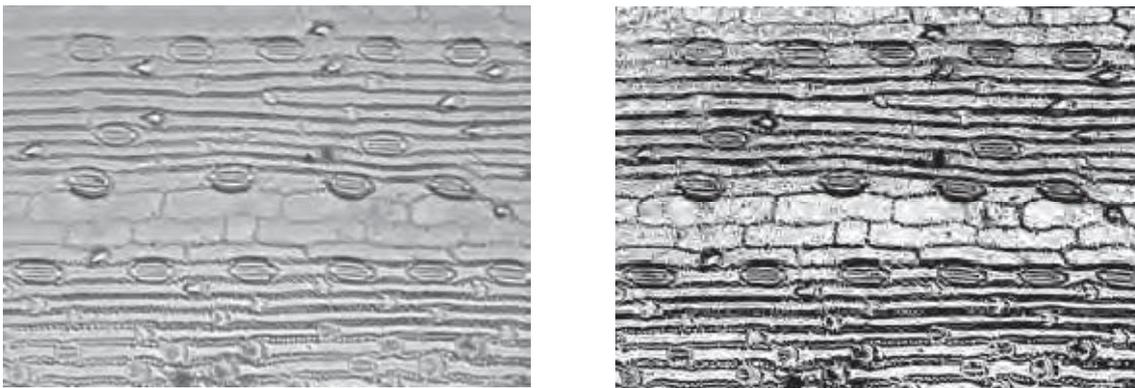


Fig 1 (a) Input image I. [6] Fig 1 (b) Result after binarization [6]

Fig 1(a) is an original image of an epidermis of a leaf. In this image we cannot easily detect stomata on it, for this purpose we have to perform preprocessing on this type of images this will remove noise in the image and make it suitable for identification and counting of stomata. Fig 1(b) shows the image after binarizing the original image. It will remove some redundancies in the original image. It sharpens the image for further processing.

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Stomata cell detection: We collect a set of n stomata templates by individually segmenting them from the images taken by using procedure described above. Pre-processing is then done using the same method explained above. We re-scale the images to fit in a defined window size of $w_t \times h_t$. In order to make the template images independent from the imaging conditions. We then rotate the original template having angles changes between $-\theta_t$ to θ_t to generate new instances. In this experiments they used $\theta_t = 14$ degrees, with an increment step of 6 degrees [6]. To detect the variation Of scale in the stomata size, In order to create new instances of template, a scale is given to the template having the limit of factor between 0.8 and 1.2 both in case of height and width direction of image. We use different scales for Height and width to collect the variability in size of stomata. The result of this stage is a dictionary of Templates of stomata having various shapes, sizes and orientations.

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Suppose $T\theta$, $\theta = (st, wt, ht, \theta t)$, is a template of size $wt \times ht$, shape st and orientation θt . Our procedure for detection of stomata region starts first by taking convolution of the image B with the templates $T\theta$ and the maximum response in a response map denoted by M is recorded at each pixel. Thus

$$Me = T\theta * B \quad (1) [6] \quad \text{Then}$$

$$M(x, y) = \max_{\theta} M\theta(x, y). \quad (2) [6]$$

The details of template can also be recorded at each pixel that gives the maximum response which is used to receive an appropriate estimation of the properties of detected stomata at every pixel.

$$\theta^*(x, y) = \operatorname{argmax}_{\theta} M\theta(x, y). \quad (3) [6]$$

An example of response map M is shown in figure 2(a) that relates to the input image shown in figure 1(a). We have observed that high response (white colours) is recorded at the pixels around stomata regions. In order to get exact detection of stomata cells, we use post-processing method on the response map M by thresholding the resulting map and reducing non maxima regions. Figure 2-(b) shows the remaining pixels were highlighted in red on the original image that correspond to candidates for stomata regions. This procedure of detecting stomata cells require setting various parameters, which are explained as follows.

- Number of templates: To collect variation in shapes of stomata cells, it is necessary to choose enough numbers of templates. In our experiments, we used six templates, as seen in Figure 2(b). However, we expect that for a different dataset, it may require a larger number of templates.
- θt and step of angle increment: in all our experiments we have used $\theta t = 14$ degrees, with an increment step of 6 degrees. In this paper, we focused on barley and wheat plants leaves where the stomata cells have approximately similar orientation. While in case of other plant leaves, which can possess random orientation of their stomata cells, the parameter θt should be increased to reflect the new conditions.
- Threshold η of response map: many false positives can produce by setting η to a small value. On the contrary to this, many stomata regions may be missing by setting it to a larger value. We have manually set $\eta = 0.5$ in the results of this paper. False positive detections can be done by using this value of η .

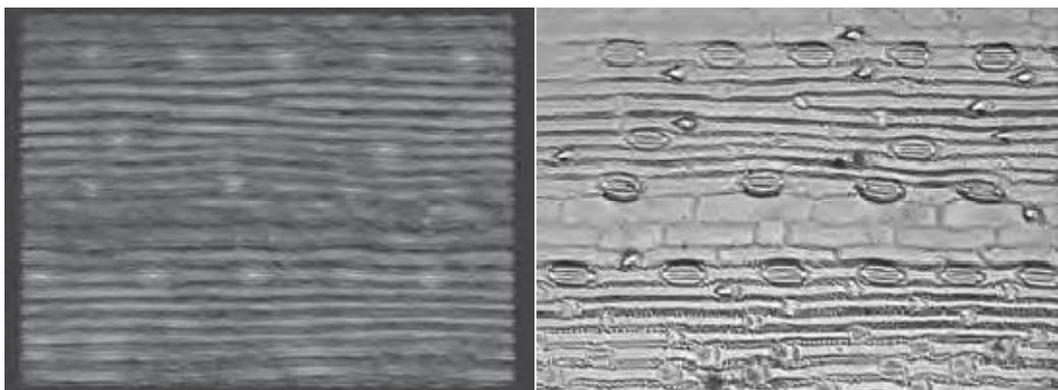


Fig 2 (a) Response map [6]

Fig 2 (b) Regions of maximum responses after Thresholding [6]

Fig 2 (a) shows the image showing response map. It shows nothing but setting of thresholding value. For this image We have set a threshold value of 0.6. This is to be done for removing false positives. Fig 2(b) shows the resultant image after applying thresholding to the earlier image. It shows the region of maximum response after applying thresholding.

2. Stomata detection and counting using Morphological operators:

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 are

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strongly concerned to concepts of Minkowski addition. The development of the mathematical morphology was originally done for binary images, and was then advanced to grayscale images. In both time and frequency domains, the main and important morphological operators are dilation, erosion, closing and opening [6]. In morphology of grayscale the dilation of f by b , denoted $f \oplus b$, is given by $(f \oplus b)(x) = \sup_{y \in E} [f(y) + b(x-y)]$, (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 $f \ominus b$, is given as follows.

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

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

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

In this method we are using two main morphological operations which are opening by reconstruction and closing by reconstruction respectively. 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] $r_f(f \oplus b)$

Closing by reconstruction is exact opposite of opening by reconstruction. It is defined as dilation followed by morphological reconstruction. The main purpose of closing operation is to fill in the small gaps in image and smoothing outer edges of the image. It is defined as a closing of f by the structuring element b after performing a morphological reconstruction can be denoted by [5]: $r_f(f \ominus b)$ (5)

By using this operation, we can easily detect and count number of stomata on the microscopic image of a leaf.

III. EXPERIMENTAL RESULTS

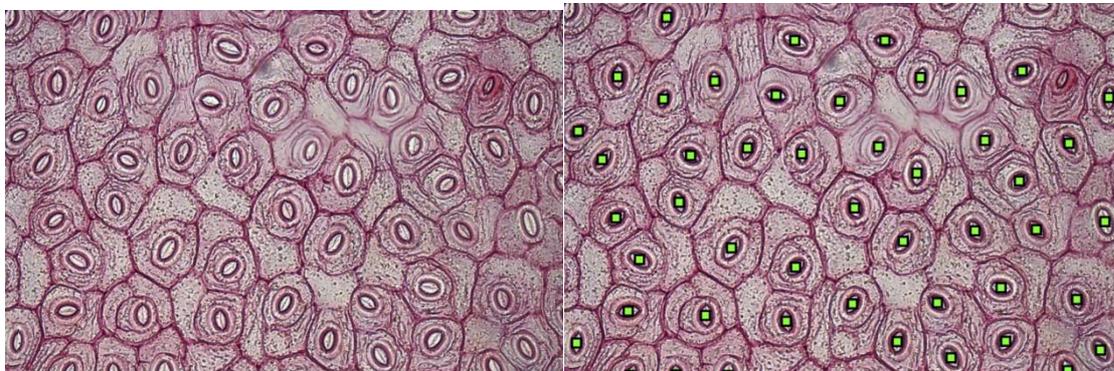


Fig.3 a) original image of species 1 [7]

Fig 3b) Image after stomata segmentation of species1[7]

Fig 3a) shows the original microscopic image of an epidermis of a leaf, performing various morphological operations like opening, closing, erosion and dilation and thresholding technique. We get the resultant image as shown in fig 3b). This image shows image which shows stomata having green points at the centroid of each stoma.

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. Fig 3b) shows the resultant image after performing all morphological operation. These images show the numbers of stomata present on a leaf image.

IV. CONCLUSION

This paper describes the two different methods of Automatic detection and counting of stomata cells the epidermis image of a leaf. These two methods are stomata detection and counting by template matching method and other is by using morphological operation. The first method stores various morphological features of images in the form of a template, then original image is to be matched with a database image to detect and count number of stomata cells on a leaf image. The second method is more efficient than first method as it required less time for computation. Thus this

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method is less complex than first one. This method uses various morphological operations to describe o stomata cell and count them. The accuracy of this method is about 94.3% [7].

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