Measurement by images of mycelial growth of fungal colonies on Petri dishes

Priscila M. Kai*, Fabrizzio A. A. de M. N. Soares*, Ronaldo M. da Costa*,
Juliana Paula Félix*, Jéssica Maria I. de Jesus†, Marcos G. da Cunha†
*Instituto de Informática, †Escola de Agronomia,
Universidade Federal de Goiás, Goiânia, GO, Brazil, 74.690–900
{priscilakai, fabrizzio, ronaldocosta, julianafelix}@inf.ufg.br, jessicamaryisrael@hotmail.com, mgc@ufg.br

***Abstract*—The use of chemicals as a form of prevention, protection, and control to combat diseases that affect plants is a feasible solution involving control measures. However, due to the emergence of microorganisms resistant to these products, new tests need to be performed in the laboratory, requiring the effort of the specialist in phytopathology and in order to investigate these microorganisms. One of the most common methods used includes laboratory tests, periodically verifying the growth of the fungal colony with measurements using ruler or caliper, but this method is imprecise. The study was developed using images containing Petri dishes with growing fungus colonies, comparing the manual method performed by the specialist in Phytopathology and by images processing. For this, processes were developed involving region segmentation by color analysis and unnecessary information removal to measure the size of colony. It was possible to verify the application and potential of the method developed for creating mobile applications. Therefore, this work presents a method for measurement of the fungal colony on Petri dishes from isolates of the fungus *Corynespora cassiicola*, using techniques involving digital image processing, with the purpose of assisting the phytopathologist professional, providing other means besides the visual analysis.**

***Index Terms*—Image Segmentation, Measurement, Fungal Colony.**

I. Introduction

Plants are essentials for human life on the planet, since they are extraction source of various indispensable resources in our day. It provides essential items, such as food, clothing, fuel, furniture elements, medicine and so on, which makes them the basis of our agriculture [1]. However, like humans, plants also suffer from pathologies, in which many symptoms manifest in their leaves, fruits, and other vegetable parts, and consequently, affecting their productivity [2] with grown and quality loose.

Diverse pathologies are occasioned by bacteria, virus, fungi, besides others, which can result damage to the plant, profit loss to the farmer, cost production raise, chemicals disease control, etc. In fact, fungus, in particular, with more than 8,000 species, are able to attack more than one type of plant, causing considerable annual financial loss. Moreover, infestation of some pathologies can vary in each plantation depending on the region's climate, soil conditions, moisture, and so forth.

Among fungus diseases, the *Corynespora cassiicola*, known as target spot, occurs in practically all regions of Brazil, provoking troubles due to resistance to fungicides, and vulnerable cultivars [2]. Although many strategies area adopted to control the target spot, such as, chemicals products, resistant cultivars, besides others, however, the infestation may not be remedied. Fungicides is further used for plant protection and diseases control, being one of the main methods for pathogens combat, and their efficiency have been improved, since World War II, with investments in research to get betters products [3].

The proper use of these products its still a viable solution in minimization damages to the crop by the destructive action of pathogens [4]. However, pathogens over time suffered alterations in the genetic structure, result in the emergence of resistant variants to fungicides [5]. This resistance acquired over the years has been one of the challenges to be fought [6]. For this, studies in the laboratory have been realized, in order to investigate isolates of microorganisms, analyzing the genetic variation and resistance of fungi to fungicides.

Among tests, circular transparent containers called Petri dish are used allowing phytopathology professionals to monitor the growing these microorganisms. Normally, monitoring of fungal colony growth is traditionally observed visually by phytopathologist, in controlled environment, through measurements using instruments like ruler (Figure 1) and pachymeter, verifying the mycelial growing periodically. In addition, the mycelial growth is performed from the approximation of area of the colony, based on measures related to the largest observed axis of colony, multiplying by the value acquired of the perpendicular axis to get the area from the mycelium.

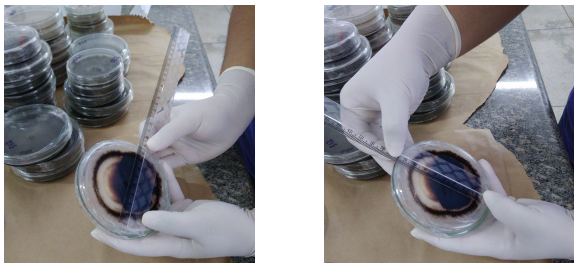

Fig. 1: Visual measurement using ruler.

However, although the visual method is most commonly used, is inaccurate, since measures of same plate can result in distinct values by different professionals, besides that, the expert's experience influences the evaluation obtained.

From this point, this paper will introduce a method to perform the measurement of fungal colony growth in Petri dishes using images with the objective of measure the diameter of fungi colonies, aiming to provide alternative methods to the professionals in relation to the traditional procedure. For this, the paper is organized as following: Literature Review in Section 2, follow by Section III with the dataset prepara-

978-1-7281-0319-8/19/$31.00 ©2019 IEEE

tion, present in Section IV the proposed method, results are discussed in the chapter V, and conclusions in Section VI.

II. Literature Review

The measurement in Petri dishes is an indispensable task for phytopathologist, however prone to errors. For this reason, search studies by robust methods are necessary, attracting the attention of researchers on the subject. Therefore, this section presents papers developed by researchers on the measurement of colonies on agar dishes.

Due to the easy availability of devices such as smartphones, the acquisition of images of Petri dishes through the use of these electronics has been present in several papers, as in [7], and in [8] using microscopy by a sensor coupled to the device. The searchers in [9] acquired images by use of a scanner to measure the survival of cells, with morphological operations based in the filter top hat in the step of image segmentation.

The searchers in [10] to evaluate the growth with studies related to solid-state fermentation (SSF), presented a method using GIMP (GNU Image Manipulation Program) for image segmentation for purpose of measure the occupation area of the colony, verifying the density of the colony through of the color intensity in images.

The authors in [11], the bacterial count was performed by color analysis, processing chromatic and achromatic images with distinct processing, by means of techniques involving threshold and grouping. Due to the colonies agglomerate, is verified the morphology of the segments detected, to acquire more precision in the counting. In [12], to measure the area of the fungi colonies, with image acquisition using a label support of size 2x2cm, made use of the statistic software RStudio with package EBImage for the segmentation of the region of the colony and area, comparing the size to the original.

III. Proposed Method

The process for measurement by images of mycelial growth of fungal colonies on Petri dishes can be visualized through flowchart in Figure 2.

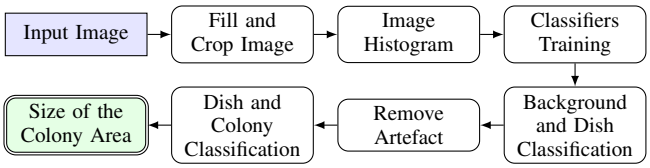

Fig. 2: Process for colony size measurement.

A. Dishes Specimen and Dataset Preparation

Images of dishes of sizes between 95mm to 100mm, containing colonies of isolates with grown variations, were acquired by a Phytopathologist, using the mobile device of the professional, photographed in the UFG Phytopathology laboratory in ambient light, with two different backgrounds. For this study, in six days not continuous were performed measures of fungi colonies sizes, taking measures from the largest axis and perpendicular axis visually observed of the colony using a ruler. Were used two types of isolates, with fast grown in the first group and slowly grown in the second.

The Petri dish images are used in input codes implemented in Matlab®, for the purpose to measure fungi colonies in Petri dishes. A size adjustment of the input images is performed with border addition, to make feasible the cropping of images in small pieces (samples) to separation in two class, the background, and Petri dish. The values for adjustment are obtained through of geometric progression of ratio equal to 2 to cropping images of size 32x32 and 64x64 pixels.

B. Histogram and Classifiers Training

In the process involving feature extraction, as input on classification models are used histograms in grayscale of samples, for the creation of input vectors of same size, built with 256 bins. To reference each class, one column at end of each vector acquired was added with reference to the group. Also, histograms were also normalized to be trained and compared with the results without normalization.

The matrix containing the histograms is loads to Classifier Learner present in the Matlab® toolbox of Image Processing and Computational Vision. Were used three different classification models, k-NN, varying the k, SVM and Trees. The output from classifier will result in two possible outputs (background and dish), resulting in a binary mask. The classifying models trained will be salved for predictions of new input data.

C. Background and Petri Dish Classification

The output of the previous process will be a mask containing an object with regions without filling belonging to Petri dish. Due to this, morphological operations of closing, dilation, and erosion were applied to smooth irregularities on object shape, involving filling of acquired shape and the remove of unnecessary noise. After this, the number of objects in the mask is checked, analyzing the connectivity of pixel in relation to neighbors, being the connectivity used of eight neighbors. The largest object is kept in the mask, and then filled.

D. Removal of artifacts

Subsequent to the process of background and dish segmentation, on the lid of the dish may contain information, such dates and also other data. Thus, the removal of these artifacts is performed not to negatively influence the next classification between colony and dish. Firstly, to identification of the artifacts is necessary identify the pixels of artifacts that stands out from others presents in the image. For this, subtractions are performed between the channels of RGB, binarizing the output by a manual threshold, and border detection. Are used two border detectors: Canny and Prewitt.

After of the border detection, the outputs are joined, resulting in the mask containing the subtract operation and border detection, and morphological operations of open, spur and clear are applied to soften the border and comprise better regions belonging to the identified artifacts. The identified artifacts will have the mask complement multiplied by the RGB image, and an interpolated filter applied in the image.

E. Dish and Colony Classification

Removed the artifacts, dish and colony classification was performed with the color-based methodology, through the selection of regions of interest, the process makes the grouping of pixels by colors, differentiating them by a pseudo-color. [13]. The image is converted to L*a*b*, and the medium color of channels a* and b* by polygon selection is calculated, to define reference markers of each class, with separation of colors by k-NN, considering the shortest distance. The output of classification will result in an array containing labels of each classified region, being the fungal colony and Petri dish.

In this study, using color-based approaches, have been developed two methods, the manual method and automatic, in which, in manual method, markers are set manually to form polygons in colony and dish regions, and a color average of the selected region is calculated. The automatic method calculates the average of channels a* and b*, performing the division of the dish in samples, calculating the mean of them. The averages will be separated into two groups. The closest pixels to the center will be selected as colony and those belonging to the dish removed, assigning the value 0 to the pixels.

F. Measuring the size of the colony of fungi

From the previous process may remain objects not belonging to the colony region, therefore is verified the distance of the centroid of the objects, selecting the object with the distance closest to the Petri dish, ending with the calculation of the average diameter of fungi colony to be compared to the measurements obtained manually by researcher in Phytopathology.

IV. Results and Discussion

The tests were performed using 110 images, containing fungal colonies in growing on Petri dishes, following the growth of 7 isolates of *Corynespora cassiicola*, causer of the target spot disease, photographed on different dates. A total of 35 images were used to create samples for histogram construction to input in classificatory models for the background and dish classification, with black and brown backgrounds. Samples were selected per resolution, containing 2400 in blocks of size 32x32 pixels and 1200 of size 64x64 pixels, manually selected.

For training of the classificatory models from histograms of images in grayscale, in HSV color model and also to normalized values, 75% of the data was defined for samples training and 25% to test. Among the samples of size 32x32 and 64x64 pixels, the accuracy obtained for the first group of images was higher in the three cases, with histograms built from samples in grayscale, grayscale + HSV, and HSV, both for the normalized data and unnormalized. Due to this, the subsequent process was performed only with images derived from the use of samples 32x32 pixels, besides presenting more details and definition in relation to object shape resulting from the classification. The accuracies of classificatory models from histograms of samples can be observed in Figure 3, where higher accuracies were observed with SVM and Bagged trees using grayscale and grayscale histograms + HSV.

Applied the trained models in new input images, the classification with Bagged trees was superior to the other models,

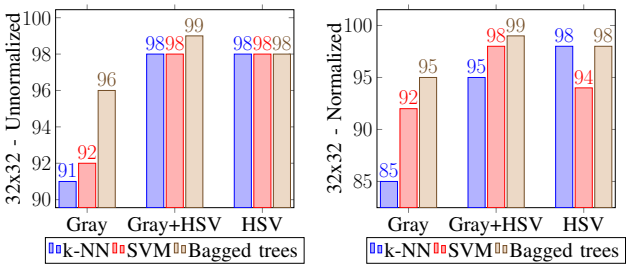

Fig. 3: Background and Petri Dishes Classification patches.

with similar outputs using data unnormalized and normalized (Figure 4), being able to identify in the majority of the dishes present in the images.

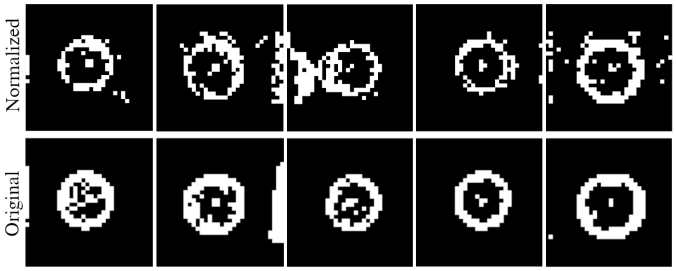

Fig. 4: Segmentation Result sample for 32x32 images.

For k-NN and SVM, the dish and background segmentation obtained with unnormalized data returned better separation of regions in comparison to normalized, with more noise and less definition of the object shape. In some images of Petri dishes it was not possible to clearly identify the regions of interest, making subsequent processes impossible. With the segment of image segmentation performed, the diameter of the plate is calculated after applying smoothing on the resulting object.

Due to some samples used for the feature extraction include background regions in cutouts made at the border of dish, the output of segmentation may contain additional area. For this, after of the dish and background segmentation, thresholds were defined from the mean color of the background pixels of an area of 100x100 pixels, with the conversion of RGB image to HSV and thresholds applied: 0,103 to 0,915 to the brown background on H-channel and pixels with values smaller than 0.4 would be replaced by 0 on V-channel to the dark background. Applied the thresholds on the HSV image, the pixels with values equal to 0 replace the pixels located in the same positions in the binary mask, with the application of morphological operations for measure the size of the colony. For the artifacts removal process, blurred information on dishes has not been completely removed.

For dish and colony segmentation, in the method using automatic selection, the result from the color channel averages was not able to correctly segmentation in dish and colony regions. For this reason, this approach was not used to obtain the final results and the manual process with polygon selection performed. Executed the dish and colony segmentation, the largest axis and perpendicular axis of colony is measured of the remaining object, comparing the measurement in pixels in relation to the size of the plate in real size, to obtain the

size in millimeters. From this, the measured data (largest axis and perpendicular axis of the colony) by the phytopathologist can be verified in the Table I, being possible to observe the variability in the growth between the colonies, of slow and fast growth. The process performed with image processing can be visualized in the Table II.

Table I: Manual measurements of fungal colony.

Data	Isolate						
	I306	I312	I313	I317	I318	I320	I323
06/06	10|10	10|10	10|10	10|10	10|10	10|10	10|10
07/06	12|11	18|21	18|18	18|18	21|21	11|10	23|24
08/06	15|13	32|34	27|27	27|26	33|30	12|11	35|36
11/06	21|20	58|59	50|50	57|55	57|58	21|19	62|60
12/06	24|23	69|69	58|58	61|65	67|67	26|23	71|72
13/06	28|27	74|75	66|65	68|69	75|75	27|26	77|79

Among the best rated dishes, the isolate denominated 306 obtained the closest measurement results compared to the manual method. The area belonging to the colony of the first day of observation in manual measurement remained the same, due to the size of the initial plug, containing the material composed of the fungus *Corynespora cassiicola*.

Table II: Fungal colony size and dish size per mm by images.

Data	Isolate						
	I306	I312	I313	I317	I318	I320	I323
06/06	9|8	10|9	9|7	10|9	10|9	10|9	9|8
07/06	9|8	21|20	16|15	24|20	22|23	10|9	19|18
08/06	12|12	34|32	22|22	30|27	39|33	–	27|26
11/06	20|19	42|37	42|32	44|44	46|44	11|12	46|38
12/06	23|23	–	50|47	53|51	58|57	13|9	55|72
13/06	30|23	58|58	53|50	57|56	–	10|9	–

Through the graph containing the relation of the measurements of each dish, performed on different days, it is possible to observe the positive relation between the pairs of measurements (manual by specialist and by images) in I306 and I313 as in Figure 5, verifies the association between the pairs of data, and the relation between the two calculated measures, linear too in the cases of isolates I312, I317 and I323. The measurements performed with isolates I318 and I320 presented non-linear relations, with discrepant measurements between the manual method by phytopathologist and by images, resulting in dispersion of the points on the graph.

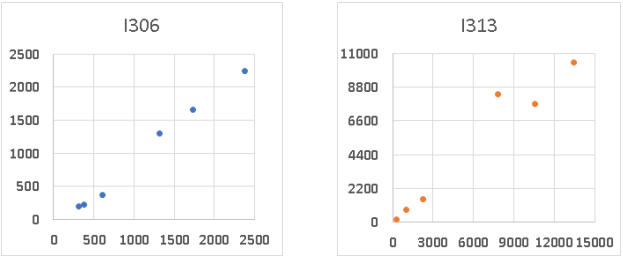

Fig. 5: Scatter graph of I306 and I313.

For images with dark background obtained better results when compared to those calculated with brown background, with less differences between measurements from the manual measurement and by images. In addition, some images present fungal colonies with shades similar to the plaque region, due to the inserted liquid (agar), with pasty appearance. This factor may have influenced negatively, both in the manual and in the automatic method using the color-based methodology.

V. Conclusions

Through images of the fungus *Conynespora cassiicola*, which causes the disease target spot, the following study was performed with the objective of measuring the diameter of fungi colonies in Petri dishes to assist professionals in phytopathology in colony size periodic measurements.

It was verified the similarity between colors of regions belonging to Petri dish and fungi colony, pointing out the need to elaborate modifications in the process of segmentation between the two regions for better results in the identification and to get more robust data, involving the measurement of the fungal colony, verifying the mycelial growth.

The study allowed to verify the applicability of the presented method for measurement of the fungi colony diameter in Petri dishes,demonstrating the potential of the method for developing mobile applications that facilitates the work of the phytopathology specialist in the monitoring of mycelial growth as an alternative to the traditional method of visual analysis which performs the repetition of measurements to obtain more accurate measurements.

References

[1] R. N. Trigiano, M. T. Windham, and A. S. Windham, *Fitopatologia: conceitos e exercícios de laboratório*. Artmed Editora, 2016.

[2] C. Godoy, C. Utiamada, M. Meyer, H. Campos, I. Lopes, A. Dias, C. Bulhões, C. Pimenta, D. Miguel-Wruck, E. Moreira *et al.*, "Eficiência de fungicidas para o controle da mancha-alvo, corynespora cassiicola, na cultura da soja, na safra 2017/18: resultados sumarizados dos ensaios cooperativos." *Embrapa Soja-Circular Técnica (INFOTECA-E)*.

[3] K. J. Brent and D. W. Hollomon, *Fungicide resistance in crop pathogens: How can it be managed?* GIFAP Brussels, 1995.

[4] E. Maranhão, J. Candeia, L. Wanderley, and E. Maranhão, "Controle químico do "mal-desete-voltas" em cebola, causado por colletotrichum gloeosporioides pens, no estado de pernambuco," *Horticultura Brasileira*, vol. 9, no. 1, p. 45, 1991.

[5] A. Garcia, "A resistência dos fungos como consequência da utilização de fungicidas sistêmicos: mecanismos de resitência, monitoramento e estratégias anti-resistência." *Embrapa Rondônia-Documentos (INFOTECA-E)*, 1999.

[6] R. Ghini and H. Kimati, *Resistência de fungos a fungicidas.* Jaguariúna: Embrapa Meio Ambiente., 2000.

[7] A. P. Martins, J. C. Pizolato Jr, and V. L. Belini, "Avaliação automática da taxa de crescimento de colônias de leveduras incubadas em placas de petri usando plataforma móvel," *Semina: Ciências Exatas e Tecnológicas*, vol. 36, no. 2, pp. 33–46, 2015.

[8] G. Zheng, S. A. Lee, Y. Antebi, M. B. Elowitz, and C. Yang, "The epetri dish, an on-chip cell imaging platform based on subpixel perspective sweeping microscopy (spsm)," *Proceedings of the National Academy of Sciences*, vol. 108, no. 41, pp. 16 889–16 894, 2011.

[9] M.-C. Biston, S. Corde, E. Camus, R. Marti-Battle, F. Estève, and J. Balosso, "An objective method to measure cell survival by computer-assisted image processing of numeric images of petri dishes," *Physics in Medicine & Biology*, vol. 48, no. 11, p. 1551, 2003.

[10] J. P. López-Gómez, C. Pérez-Rivero, and C. Webb, "Investigating a non-destructive alternative for a preliminary evaluation of fungal growth in solid state fermentations," *Journal of Microbiological Methods*, 2019.

[11] C. Zhang, W.-B. Chen, W.-L. Liu, and C.-B. Chen, "An automated bacterial colony counting system," in *Sensor Networks, Ubiquitous and Trustworthy Computing, 2008. SUTC'08. IEEE International Conference on*. IEEE, 2008, pp. 233–240.

[12] F. O. Silva, A. T. Itako, and J. B. T. Júnior, "Mycelial growth assessment by digital image analysis in r software environment," 2017.

[13] U. Qidwai and C.-h. Chen, *Digital image processing: an algorithmic approach with MATLAB*. Chapman and Hall/CRC, 2009.