APPENDIX - I

ADDITIONAL WORK RELATED TO BIOLOGY

I.1 Count colonies by using ImageJ – Colony Counter

Now we will use the ImageJ software to measure the size of these bacterial or mold colonies. ImageJ which is an open source software anybody can use it doesn't cost anything and it is very powerful and that's why we use it. If somebody want to get it just click on download (https://imagej.nih.gov/ij/download.html) of course and you can download for Linux or Apple or Mac and apple or Windows and whatever bit like somebody using 64-bit window so use this one it runs on Java just download it and you don't have to install it just unpack it and it runs and all you have to do at least for window is just find this dot exe and just double click it or just make a shortcut to it on desktop so either way that just launches the program and in seconds it will start.

Now for counting colonies in a petri dish just go to file open and I've taken a picture of one of the petri dishes and you can see some colonies on here it's on a dark background which helps the colony show up. After that take a ruler in the picture with the Petri dish this is critical because you need to use this ruler to calibrate the software, once you calibrate the software you can determine the area of these colonies in square millimeters but you can only do that if you have something of known size in the picture that's why we always put this ruler in the picture so what I am going to do is I am going to take this straight line and I am going to click and drag it a known distance and in my example here I am going to click and drag it 10 millimeters and you can go as long or as short as long as you know the distance. Alright so now we know that this red line here is exactly 10 millimeters so let's tell the software that what is going to analyze set scale this window pops up and you see it is measuring the distance and pixels you know that that is a known distance of 10 and the unit is millimeters and now when you hit OK now the software is calibrated and so you could redraw another line and that should be 5 millimeters and if you hit ctrl M it presents the length is 5 MM, so now our software is calibrated.

Now for measurement just draws an oval tool around the edge of the Petri dish to select the Petri dish conations the colonies and adjust as per the shape of the Petri. Select the region that doesn’t include edge of the dish. Now select→edit→clear outside to show the Petri dish only. Convert the input image from 24 bit to 8 bit if necessary. Now select → adjust → threshold to obtain the thresholding process. Adjust the effect
while the cells can be visualized clearly. For that adjust the sliders appropriately. Count cells by Analyze \rightarrow Analyze Particle. Set minimum and maximum size of cells to be detected. Set circularity (means the roundness of the object) accordingly. Now at the time of selection of selection of outlines mainly three options will be displayed. Now in the screen you can see the outlines of every colonies separately by numbered also. At the same time on the screen in other window a list of all colonies presented with min max and area separately. Now number of cells can be extracted in summary dialog. Detailed information for each counted cells are shown in Results dialog. We can save this information either in .txt or .csv format. Sometimes some of the colonies are overlapping and in that case add watershed to identify the overlapping features and to remove the occlusion form the colonies. For that simply take any binary image click process and then watershed, so it will simply parts out all the regions for easy calculation.

![Image](image.png)

Figure I.1 Imagej – Count number of colonies in a Petridish

### I. 2 Calculate Leaf Area with ImageJ

ImageJ is an open source image improvement processed program designed for scientific multidimensional images. ImageJ is highly extended, to perform various types of
functions, huge plugins and scripts and wide user community. ImageJ is the world's fastest pure Java image processing program. Powerful resources such as script editors and personalized update sites help you develop and share reproducive analysis workflows. ImageJ is written in Java, which allows it to run on Linux, Mac OS X and Windows both in 32-bit and 64-bit mode. ImageJ and its Java source code are freely available and are in the public domain. No licenses needed there's a huge and knowledgeable universal user community in ImageJ. More than 1800 users and developers subscribe to the ImageJ mailing list. Using macros, we can automate the tasks and create custom tools. Generate macro code using the command recorder and debug using macro debugger. More than 300 macros are available on the ImageJ Web site. Extend ImageJ by developing plugins through ImageJ created in Text Editor and Java compiler. More than 500 plugins are available. Use the image to develop applets, salons or apps such as image processing toolkit (class library). It can filter the 2048x2048 image in 0.1 seconds. It's 40 million pixels per second! 8-bit grayscale or indexed color, 16-bit signed integer, 32-bit floating point and RGB color. Open and save all supported data types as TIFF (uncompressed) or raw data. Open and save GIF, JPEG, BMP, PNG, PGM, FITS and ASCII. Open Decomplet Open TIFF, GIF, JPEG, DICOM and raw data using multiple plugins, open up many other formats and save zoom tools (1:32 to 32: 1) and scrolling images all over any magnification of analysis and processing functions. Factors work on.

Create rectangles, oval or irregular area choices. Edit line and point selection preferences and automatically create it using the stick tool. Select, fill, clear, filter or select. Save preferences and transfer them to other images. 8-bit grayscale and RGB color images support leasing, sharp, edge detection, mid filtering and thresholding on both. Arrange the brightness and contrast of 8, 16 and 32-bit images efficiently, crop, measure, resize and rotate vertically or horizontally. Size area, mean, standard deviation, minute and maximum selection or overall image. Measure length and angles. Use real world scale units such as millimeters. Arrange using density standards. Create histogram and profile plots. Split 32-bit color image into RGB or HSV elements. Merge 8-bit components in color-image convert an RGB image to an 8-bit indexed color. Apply pseudo-color palettes to grayscale images. Show "stack" of related images in a single window. Process the entire stack using a command Open the folder of images as a stack. Save stacks as multi-image TIFF files.
**1.3 Calculate Leaf Diseased Area with ImageJ**

Here this appendix explains how imagej will be helpful for finding the leaf area and the diseased area. Let's open the image by simply drag the image to the software or you can go to file then open it by selecting from where it is located. Before doing any analysis we need to convert it into 8-Bit mode, Go to Image → type and then click on 8-Bit. But, before going to analysis the system does not know about the scale of the image that's why the image need to be calibrated or the scale should be set. How to set the scale Select the magnifying glass option here and click on the scale bar already scanned along with the image. Zoom it in till it is sufficient and then select the straight line. Draw a straight line over the scale bar The straight line is drawn go to analyze and then set scale The dialog box appears. It's showing the pixel 101.25 that is the selected one. Known distance it is showing as 0, but actually is 300nm Let's write it as 30 mm. Now our image is calibrated and ready for any analysis. Follow the steps for image analysis. For stepwise understanding follow the figure I.3

1. First Open the Image J software.
2. Then, open the required image which is to be analyzed.
3. Please take note: you have to take the image with a calibrated scale. Now, we will calibrate/set the scale of the image.
4. Convert scanned color image of leaf to grayscale: Image → Type → 8-bitYou can also smooth the image.
5. Threshold the leaf image using the automated routine:
6. Process → Binary → Make Binary
7. Please follow the subsequent steps. Then go to "Adjust" and then go "Threshold".
8. Follow the steps. Please remember that, here we only want to calculate the area of the diseased portion. Then, you have to select the required area by selection tool.

9. Calculate area of green portion:
   Surround the leaf with the rectangular selection tool: Analyze → Analyze Particles

10. Then go to "Analyze;», then "Tools" and then "ROI Manager". Follow the steps.

11. Then go to "Measure in the "ROI Manager" window.

12. Here, the calculated area is in cm². Do the same at least 4-5 times, then take the average value for further calculation.

13. Don’t forget to save the data. Follow the steps critically.

14. (a) Original Image (b) 24 bit to 8 bit image - Gray scale (c) Binary Image (d) Threshold Image (e) Scaled image for diseased area calculation

Figure I.3 Imagej – Diseased Leaf area Calculation
I.4 Colour Image Segmentation

In many applications of computer vision and image analysis, colored image segregation methods for colored images are becoming more popular. This appendix introduces auto-segmentation based on light. By implementing a fast-shift method with initial parameters, this method segments get the result of an optimal analogy between the output image and the inseparable image by replacing some importantly moving dimension values by automatically using the image in the final area, using a colored region to speed up the image. To reduce the number of colors in the image that will be used in comparison, a radicalization process is applied to the original unrelated image. Changing values in the polar values rather than using a specific value makes the proposed algorithm flexible and strong against different image features. The effectiveness of the proposed method for various images, including various objects of metals and electrics, is examined in experiments.

Color strips are an important tool for color picture analysis, because it is the starting point of various types of technologies such as qualification or indexing. This paper color represents a new method for automatic construction, which dynamically locates its number according to the image's visual content. This method correctly divides the HSI color space, which is obtained by partitioning the histogram individually associated with each color component. As a result, we get the hierarchical color, which represents the colored image with a small number of colors. In this experiment, the image of the input color will be presented vaguely in 5 boxes. Vague representation uses spatial information from the histogram-based windows process. K-bins are used to cluster brighter image data.

![Image](image_url)

Original Image  Gray Image  Colour Segmented Image

Figure I.4 Colour Image Segmentation

Here the above figure I.4 shows that after taking the original image the image has been applied coarse function and image got converted in to a coarse image and then on the coarse image the k-buins algorithm applied by using the k-means method already explain in the research report earlier and finally the gray scale image will expose
number of possible colours in my case the number of colours are 5, so it will give us five different colours from the image. Here the diseased portion coloured by yellow colour and the dark brown dots are coloured by red colours and by this way the diseases can be identified in a simpler way.

We have introduced a novel color reduction algorithm that allows a user to create a common color for flexibility to add multiple images, transparent alpha images and color palette. This method was widely tested with artificial and natural images and the results are reported here. Experimental results show that the proposed method produces excellent results and exhibits current state-of-the-art color reduction methods. The proposed algorithm can be used for digital broadcasting applications. Colored color names obtained for providing qualitative image descriptions can be combined with the dictionary. Such qualitative image descriptions can be used for image retrieval from large image databases. The proposed method reduces color information significant information, either this method is considered as a pre-processing module for color image segmentation or local information can be included in the proposed method and direct segmentation results can be achieved. The proposed method can be used to create maximum thumbnail view images for web design. Representing a set of images with a common palette significantly increases the futility of pixels in the image and thus reduces the bandwidth needed for broadcasting images on the web. This feature can be included with image coding and transmission.
## APPENDIX – II

### PUBLICATION DETAILS

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<td>1</td>
<td>Estimation of Plant Leaf Area using Java Image Processing Techniques</td>
<td>International Journal on Recent and Innovation Trends in Computing and Communication ISSN: 2321-8169</td>
<td>2015</td>
<td>Volume:13 Issue: 1</td>
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<td>2</td>
<td>Study Of Image Processing and Pixel Identification for Citrus Leaf Diseased Portion</td>
<td>International Journal of Engineering Research ISSN (Print) : 2347-5013</td>
<td>2015</td>
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<td>3</td>
<td>Study of Detached (Fresh) Leaf and Dried Leaf with Image Processing</td>
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<td>5</td>
<td>Analysis of plant leaf area using Java image processing techniques - Scaling and non scaling</td>
<td>Ecology, Environment and Conservation ISSN: 0971-765X</td>
<td>2016</td>
<td>Volume:22 Issue: 2</td>
<td>NAAS Rating: 4.89</td>
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<td>7</td>
<td>Analysis of image processing in the field of agriculture for the work of classification of plant leaf diseases</td>
<td>Gujarat Journal of Extension Education ISSN 2322-0678</td>
<td>2017</td>
<td>Volume 4</td>
<td>NAAS: 3.86</td>
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<td>8</td>
<td>Classification of citrus leaf diseases by image processing based on texture (Statistical) related features</td>
<td>International Journal of Chemical Studies Online ISSN: 2321–4902, Print ISSN: 2349–8528</td>
<td>2018</td>
<td>Volume:6 Issue: 5</td>
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# APPENDIX – III

## CONFERENCE DETAILS

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