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Microscope image matching in scope of multi-resolution observation system

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Abstract

Multi-resolution image processing are part of this concept that has a purpose to extracting the detail information of the multi-scale input image. However, in general, to process a multi-scale image there are issue that need to be solved, for an instance color difference, image matching failure, and lack of data which can be observe in a same time. In the same way, observation are work where object of interest are being analyze and being perceive. Observing a multi-resolution image with a lot of object interest would be a daunting task to do. There have been many attempts to match and stitch two or more images into one but still has a chance to get failure. In this article, the author propose a multi-resolution image observation system which are focus on three improvement field successfulness rating of image matching, utilize a classification algorithm to help perceiving object interest and user-friendly interface viewer. On pre-processing, this system take a four type magnification level of image; 10 ×, 20 ×, 50 ×, 150 ×. By investigating image matching parameter to stitching group of images, handling false positive of feature point occurred, and then finally arrange and show group of synthesized images inside web application, we believe user can observe microscopic image in a cushioned way.

Introduction

Concept or idea of multi-resolution are broadly applied and prolific to solving complex problem that unable to be solved in just one resolution. The demand to explore and analyze an object are grow from one fix image resolution into multi-resolution image in a same object being used together. The principal use of multi-resolution are to search image structure for object detection, registration, image tracking object, analyze large digital arrays, and even on feature detection image processing. Plenty of research are born from this multi-resolution idea for an instance pyramid algorithm which using multi-resolution architecture to reduce computational complexity [3], multi-resolution approach to image processing analysis [5], color segmentation [9], segmentation technique using extrema in images [7], and multi-resolution microscopy [11]. There are one development of multi-resolution system [13] which inspired this research to be conducted. This work are took some problem to be solved on regarding to extend basic capability of microscope in electronic industrial.

Generally microscope on electronics things will be used when comes to inspecting and observing tiny object on electronic boards, path, or component. For a small case it was easy to recognize and understand where is the interest place and object which need to be interact by the observer, then if there are some component are broke and observer know the place of it, observer will give an action. However, if this case will be applied on a pretty large ROI with a hundred of components that slightly different, it will raised a problem of recognition. Time consuming are relatively could be solve by upscaling specification of the hardware capability, however if we don't concern with how process in a system work, bad system can be an obstacle too. A system can be considered to be bad if the process from input until output are neither not user-friendly nor bring a bad performance. In this paper we using two well-known language such python and javascript not just because of their popularity but more than that because of their specific capability to solving a particular problem. Python are developer friendly yet robust in image processing field. In other hand, javascript are part of the web framework development which has the core to make website application more human and friendly. Both of these language will be used by the write on this paper to develop a multi-resolution system especially to observe a microscopic image object.

The broad objective was to develop multi-resolution images synthesis in microscope image observation system which can show multiple resolution image synthesis with an user-friendly interface and recognize the object interest of the image synthesis. First, this system have to be feed with same set of object image in a difference acquisition condition type. In regards to enhance part of Suzuki's work [14] which on false positive possibility of image feature matching, we conduct experiment with main parameter of image feature matching. This experiment will conduct inside pre-processing phase in this system. This pre-processing system will contain several method for an instance image feature detection, image interpolation, image morphology, hamming distance (HD), RANSAC, and color brightness tuning.

Second, still on a same phase of pre-processing, after image synthesis already generated then we need to train the object interest within image synthesis. Bag of words (BoW) as an image classification, part of Neurolinguistic algorithm (NLP) which was developed by Csurka et al [4] will help this system to recognize by creating vocabulary that can describe the image terms. With BoW, microscope capability are not just to magnify the object but also recognize the object interest in multi-resolution scale.

Third, post-processing will be conduct. In this phase we arrange and show synthesis image inside web application powered by javascript. We focus on feature to showing two different range of interest (ROI) in a same windows. By showing two ROI of synthesis image in a same windows, we can investigate an object image in both far and near perspective. On This work will try to improve my last work too [12] in a context of zooming transition and resolving overshoot.

Related work

Hamming distance

Hamming distance (HD) is a number represent the minimum number of errors between two strings of equal length. There are project [10] which applying HD as a learning metric. In this work we need to compare two images which is one is the original and the two is the synthesis image. We want to measure how far the difference between these two images. At the end, the smallest distance means the least difference will be taken and processed regarding to arranging multi-resolution system.

Bag of words

The literal meaning bag of words (BoW) is a method based on vector quantization of affine invariant descriptor of image patches. BoW consist with histogram of the number of occurrences particular image patterns in a given image [3]. In early development, BoW has been called Bag of keypoints regarding this method purpose was to confront generic visual categorization. On the end of pre-processing phase, BoW will be used to develop a vocabulary which can describe the object interest found in image synthesis. There are four things need to do to recognize object interest in image synthesis: clustering, bag of visual words, generating vocabulary, and data training. Start with clustering which in this work we used k-Means clustering[8].

Feature detection

In the first of pre-processing phase we need to match images to become synthesized image. In order to do that, we utilizing local feature detector like SIFT and SURF, however we need to know which one can fit with this system. As Bay et al mentioned in his paper [2] SURF are more faster than SIFT, but how with the performance dealing with ratio and RANSAC reprojection error? Here we did two experiments to compare SIFT and SURF performance, based on time (Fig. 1) and based on occurrence of false positive (Fig. 2).

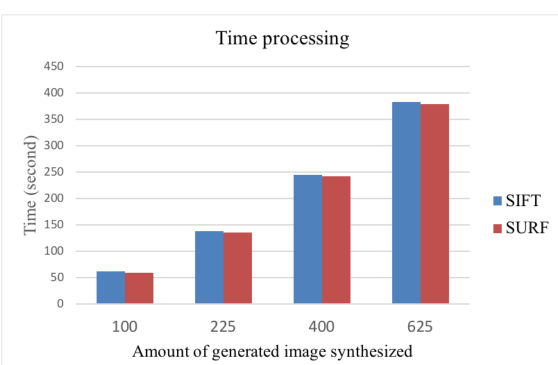

Figure 1: amount of generated image synthesized

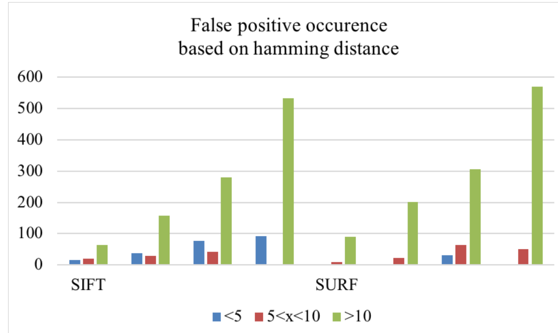

Figure 2: Experiment result of false positive occurrence on SIFT and SURF measured four times using HD.

Image morphology

Image morphology is a collection of non-linear operations related to the shape in image. One of morphological transformation are called dilation and erosion and both of these are widely used in a context like noise removal, isolating an element, and joining separate element. In this work, we found while stitching two image together, the end result generated synthesis image producing black line seam between two stitched image. This is happened because size of hole on image mask are same with size transformed image. In order to eliminate black seam on synthesis image, we use dilate image morphology to make hole little bigger than transformed image.

Hamming distance

Hamming distance (HD) is a number represent the minimum number of errors between two strings of equal length. There are project [10] which applying HD as a learning metric. In this work we need to compare two images which is one is the original and the two is the synthesis image. We want to measure how far the difference between these two images. At the end, the smallest distance means the least difference will be taken and processed regarding to arranging multi-resolution system.

Material and device

Data material that will be used in this research will correlated with the industrial object. Limitation of using specific data material are applied on this research like we did not used image texture with reason it will confusing for image matching method for looking the feature point. We use Edmund USAF 1951 1X as data material. Even though the data material are similar, we divide as two group from this data based on the content density of the image. First type are image with more content of details, much enough but there still background image that can be found. The second type is image with lesser content of details, we can distinguish clearly what is object focused what is background image.

Table 1

Dimension	1024 × 768
Colour model	RGB
Alpha channel	No
RAM	16GB
VGA card	NVIDIA GeForce GTX960
Processor	Intel(R) Core(TM) i7-4790 CPU @3.60 Ghz
Magnification	10×, 20×, 50×, 150×
Numerical aperture	0.3, 0.46, 0.95 , 0.95
CCD camera	1/3 colour image sensor 100W halogen lamp, spectral irradiance = 350 - 700nm
dFOV	165 µm, 3.1 µm, 0.35 µm, 0.2 µm

More detail about table 1 wrote above, acquisition data instrument that will be used is Laser scanning microscope from Keyence VK-X200 utilized with four objective lens, 10×, 20×, 50×, 150× magnification [16] as Fig. 3 showed below. Four images will generated in 1024×768 pixel dimension taken by optical colour camera CCD, with light resource 100W halogen lamp (spectral irradiance = 350 nm-700 nm). As Tkaczyk said [15] limit of light microscopy measured by wavelength are around 100 nm-380 nm and our object image will be around 300 nm, therefore VK-X200 are adequate. We mainly process the whole algorithm by python but we used a viewer using javascript too. The main reason why we using these two languages is because of their specification and merits, python are robust on data scientist and javascript are well supported and can bring the good user-friendly interface in a software.

There are two case of image matching so far, case (a) I(x,y) which within one of it is spatial area included J(x,y) area, and case (b) where image I(x,y) and Y(x,y) have overlapped dot each other. We will focused on the case (a) because we using 4 types magnification which is we have multi-resolution in it. Type (b) are stitching between the same resolution, so we will do this type in future work.

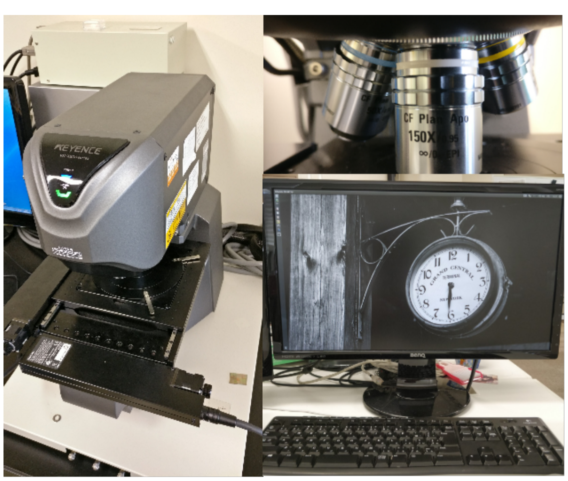

Figure 3: Acquisition device from left, Keyence VK-X200 with four magnification type lenses (10×, 20×, 50×, 150×), and personal computer.

Method, results, and discussion

Pre-processing: Image synthesis

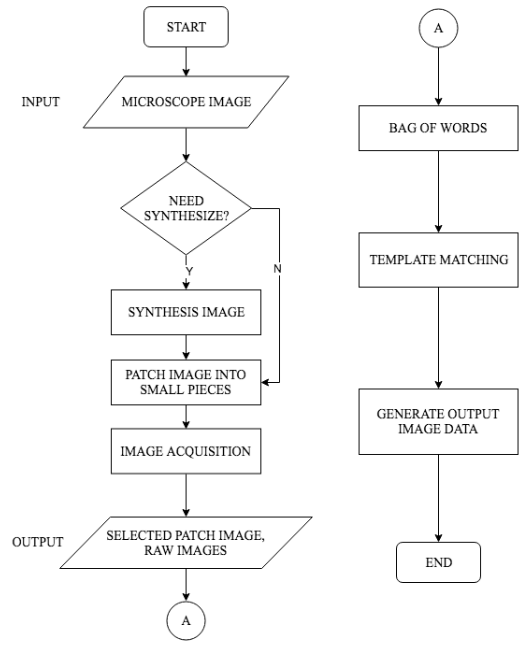

Figure 4: Propose pre-processing system flowchart. Multi-resolution image synthesis (left) and learn flow of patch images data using Bag of words (right).

There are two phase processing: pre-processing and post-processing. In figure 3 we can see two region flowchart, in the left is multi-resolution image synthesis phase and in the right is BoW to learn patch image data feed from multi-resolution image synthesis phase. Both of these sub pre-processing here has a purpose to generate synthesis image which their object of interest can be extracted so that system can learn to recognize the data train. At the end, user would not have a hard time to find and recognize the object interest, because this system will give an information of location and classification of the object interest right away.

First phase is multi-resolution image synthesis. First we need to acquisition microscope image by using four type lens. This intentionally to make a learning variance of SVM. Image taken are the same object but taken with different lens.

After set of microscope images was taken, system will give two option, whether set of image will being synthesize or not. This phase will fully depend on user need, whether want to synthesize or just let the set of image untouched. If user need to synthesize, then set of images will treated with feature matching point algorithm.

After image has been synthesized then continue to train image data phase. This will do by manually cut object interest in image synthesize which on data learn phase will be feed up in order to gather a vocabulary.

Black seam problem

When two images has been stitched, there still black seams happened between edge of image reference with image source. This happened because size of black mask are perfectly the same with transformed image. Fig. 5 will explain how black seam occurred when synthesizing image.

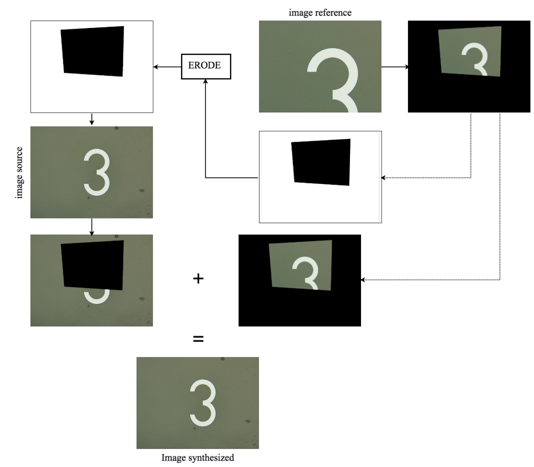

Figure 5: Flow to remove black seams

Basically to solving black seam problem is to utilize common morphology operators, erosion. There are another one called dilation but in this case we want to make the mask little bit narrower than the transformed image so when it will be stitched black seams will not occurred. Erode operation basically compute a local minimum over the area of the kernel. This operation erode the source image using formula below.

$$dst(x,y) = \min_{(x',y')\,:\,element(x',y') \neq 0} src(x+x', y+y') \quad (1)$$

As formula 1 above, the function erodes the source image using specified structure element that determines shape of a pixel neighborhood over which the minimum is taken.

False positive problem

On feature method like SIFT or SURF, there are mismatch error called false positive mostly happened because of pair feature point between two images are not the proper place. This is happened because of overfitting or underfitting feature point data set. Below in figure 15 are the example of combination between r and threshold in order to find best fit between two images. As we can see in Fig. 6 which is taken from 10× magnification image and 20× magnification image, best fit (a) and over fit (b) are have pretty close similarity from the r and threshold value. However, in (b) has several wrong pair (red dashed rectangle) occurred which leading to image distortion (b). By this experiment, we be able to get two extreme value of best fit and worst fit (overfitting or underfitting).

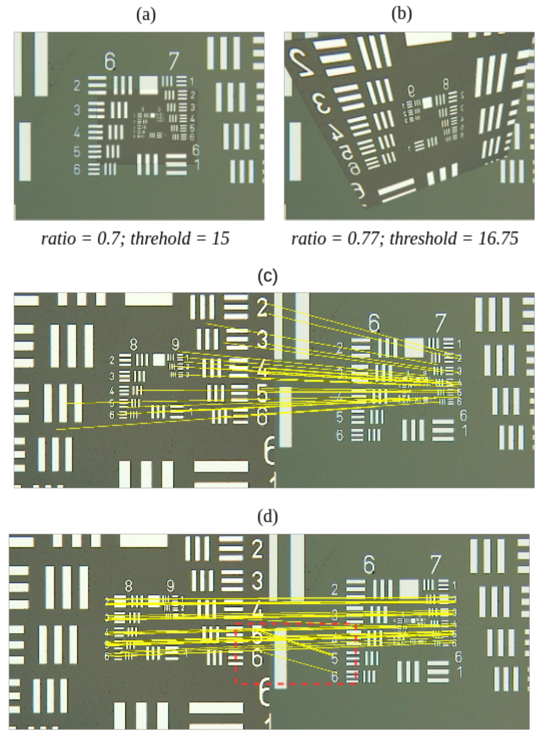

Figure 6: (a) Synthesized image with proper ratio and RANSAC reprojection error. (b) With no proper ratio and RANSAC reprojection error. Picture (c) show the proper pair match keypoints instead picture (d) has misplace pair keypoints

Image synthesis experiment

As Fig. 7 show below, we arrange experiment from two controlled variable (distortion and black seam) and two dependent variable (r and threshold). We using permutation of the two controlled variable which is n controlled variable, $n_2 = 4$ permutation in every data set I. We want to figuring out how big the impact of distortion and black seams on this system with object prediction. The r and threshold value are being taken from the experiment 4.1.3 which is we are looking for product of the best r and threshold and the worst of r and threshold value. For an instance, if the distortion value is No then we use $r = 0.73$; $threshold = 19$. If the distortion value is Yes then we use $r = 0.47$; $threshold = 20$. This case we use on $I'_{10_{2}0}$.

Pre-processing: Data learning

After image synthesis has been generated, next method is BoW which have main purpose to help detect a specific interest object. The basic things of BoW are already explained in related work above, first we have to understand what feature do we need to be learned. Here we pick object from synthesized image which generated from our system, and then put it manually on a folder with the same type of another data that we already pick. Then we build a vocabulary, registering the feature points with the dictionary which we create. The last part is to train the data with SVM. After that we serialize the learned SVM model for future use.

No.	Data set	Distortion	Black seam	r	Threshold
1.	I'_{10_20}	No	No	0.73	19
2.		No	Yes	0.73	19
3.		Yes	No	0.47	20
4.		Yes	Yes	0.47	20
5.	I'_{20_50}	No	No	0.70	15
6.		No	Yes	0.70	15
7.		Yes	No	0.30	12
8.		Yes	Yes	0.30	12
9.	I'_{50_150}	No	No	0.78	22
10.		No	Yes	0.78	22
11.		Yes	No	0.53	10
12.		Yes	Yes	0.53	10

Figure 7: Table image synthesis with two controlled variable(the existence or not of distortion and black seams) and two dependent variable (r and threshold).

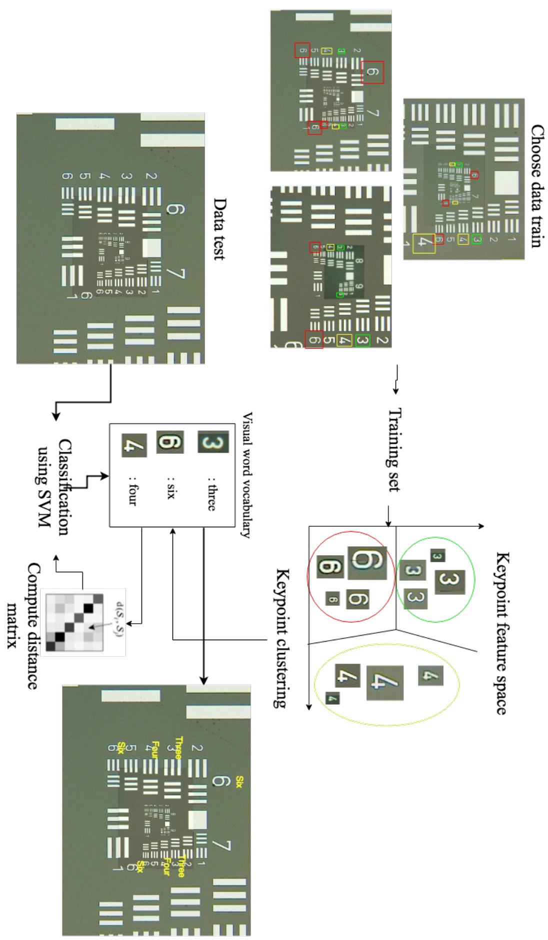

Figure 8: Bag of words process flow

First on image set $I = (I_{10}, I_{20}, I_{50}, I_{150})$ as the input images in the beginning, set of image synthesize $I' = (I'_{10}, I'_{20}, I'_{50}, I'_{150})$ are generated. After that from I' we make several patch images from each of I' to supply the data train. Patch images from each of I will be classified by using k-Means classifier. Classifier that we found on each of I are depends on what interest object (vocabulary) that able to pick, therefore the classifier in every I are different. Here the table of interest object that we found on each I.

Type I'	Vocabulary
I'_{10_20}	$(1,2,3,4,5,6,7,8,9)$
I'_{20_50}	$(1,2,3,4,5,6,8,9)$
I'_{50_150}	$(1,2,3,4,5,6,8,9)$

Table 2: List vocabulary of I

We need to train and test our patch images data from synthesized image by using k-Means classification and SVM as the supervised learning models in order to make the multi-resolution system can perceive the interest object inside synthesized microscope image.

After we build vocabulary from three of I images and classify it into several classes as table 2, we do an SVM classification. Every patch images will be extracted and every feature point found will be fed into a SVM dimension. Visualizing feature point dimension in a plot are hard to do, therefore we look for another way to proof the effectiveness and correctness of how our SVM works in this system.

To make sure that our classification model are reliable enough, we need to measure the effectiveness of the model by using confusion matrix. Confusion matrix is a table that able to describe the performance of classification model on a set of data which the true values are known. There are True Positive (TP), False Positive (FP), False Negative (FN), and True Negative (TN). TP means what we predict are well proven positive and true. TN means what we predict are negative and its true. FP and FN are simply two kind of errors which mean our prediction are incorrect.

Predicted Values \ Actual Values	Positive (1)	Negative (0)
Positive (1)	TP	FP
Negative (0)	FN	TN

Figure 9: Confusion matrix

$$Recall = \frac{TP}{TP+FN} \quad (2)$$

$$Precision = \frac{TP}{TP+FP} \quad (3)$$

$$accuracy(y, y') = \frac{1}{n_{samples}} \sum_{i=0}^{n_{samples}-1} 1(y_i = y'_i) \tag{4}$$

In confusion matrix there are a lot of prediction measurement method that we can gather, however in here the most common metric that people always used is precision and recall. Precision are how much we can predict correctly, from out of all the classes. Precision formula are shown on formula no. 3. In other hand, recall has the slightly the same meaning with precision, but have a different on the classes where recall predict out of all positive classes. The formula of recall is on formula no. 2. However, it is difficult to compare three model from our case with low precision and high recall or vice versa. Therefore we need F1 score (9) to help measuring recall and precision value at the same time fairly. F1 score is the average of the precision and recall, where the best values will reach 1 value and the worst value will reach 0 value. Here is the F1 score of three I image synthesis. From table 3 we can conclude that I'_{50_150} has high score which mean in I'_{50_150} all of the data test gave a correct prediction.

Type I'	F_1 Score
I'_{10_20}	0.804
I'_{20_50}	0.64
I'_{50_150}	1

Table 3: List F_1 score of I

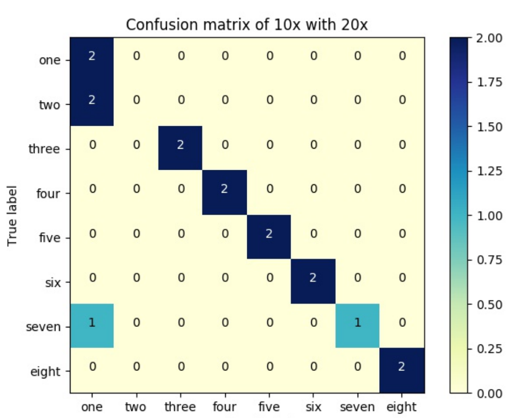

Figure 10: Confusion matrix of image synthesis 10 with 20 image magnification.

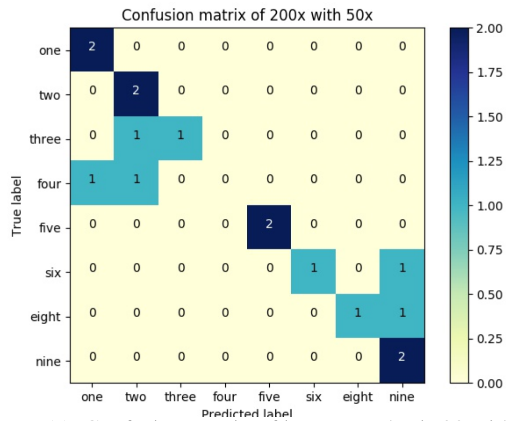

Figure 11: Confusion matrix of image synthesis 20 with 50 image magnification.

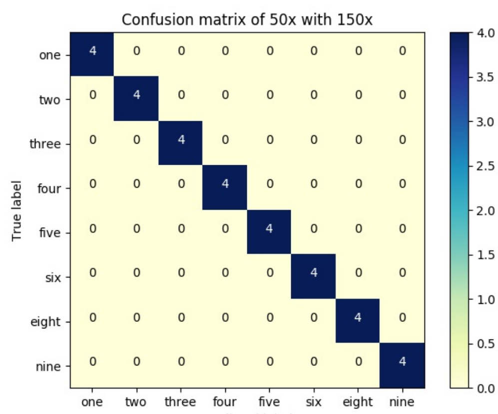

Figure 12: Confusion matrix of image synthesis 50 with 150 image magnification.

As we can see in confusion matrix Fig. 10, 11, and 12 above, we can analyze that there are FN and FP occurred. This happened mostly because of several feature point has been owned by more than two classes. To solving this kind of problem, we need to play with the C-parameter on SVM which is control the hyperplane to be draw between the classes. High C-parameter will prioritize effectiveness, converse with that low C-parameter will loose.

Post-processing

As Fig. 13 shown below, the order of post-processing phase will start by fetching image data, then continue to view the images with magnify features. In fetching image phase, file that will be fetch are in a JSON file, so we need to parsing the JSON file to understand the structure of the generated synthesis images.

To show package data of pre-processing phase, it is need to show the images and the data of recognition from BoG with graphical user interface (GUI) and we decided to build the system using web application. The web framework mainly consist of several programming language for an instance Javascript and packed with standard markup language called Hypertext Markup Language (HTML). HTML play the part of making the skeleton of the web application. And last but not least is Cascading style sheet (CSS) which have a role to control HTML elements in a screen. The GUI features consist of three parts:

1. **Select path file**, first we need to know the path of the files of our image synthesis which produced by pre-processing phase mentioned above. The number of image that will be shown will depend on the Javascript object notation (JSON) which go along with the images. This JSON file literally are structured text file which describing the structure of the multiresolution images synthesis.
2. **Mipmap**, will be shown as many as level of magnification generated. Mipmap are simply representation of real image object in smaller size.
3. **Viewer**, will be shown as many as level of magnification generated. Mipmap are simply representation of real image object in smaller size.

First prototype of this web apps are done, Fig. 14 showed sample of the screenshot image while magnifying sample image in different ROI. In part (a), (b), and (c) on the top area are the mipmap of the image which being observed then the bottom area are the magnified area.

Color difference or brightness difference is a condition which illumination value in an equal part of both image are different due to using a different type of lens. This occurred because of lens diaphragm between high magnification lens collect more light than lower magnification lens. We want to know how impactful color difference to contributing false positive in feature matching. Beside of that, we try to minimize the brightness difference between two images by using brightness average. For basic calculation we using average formula below.

$$B_d = B_{ave1} - B_{ave2} \tag{5}$$

$$I' = I + B_d \tag{6}$$

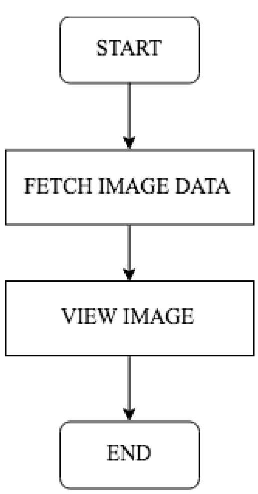

Figure 13: Post-processing flow

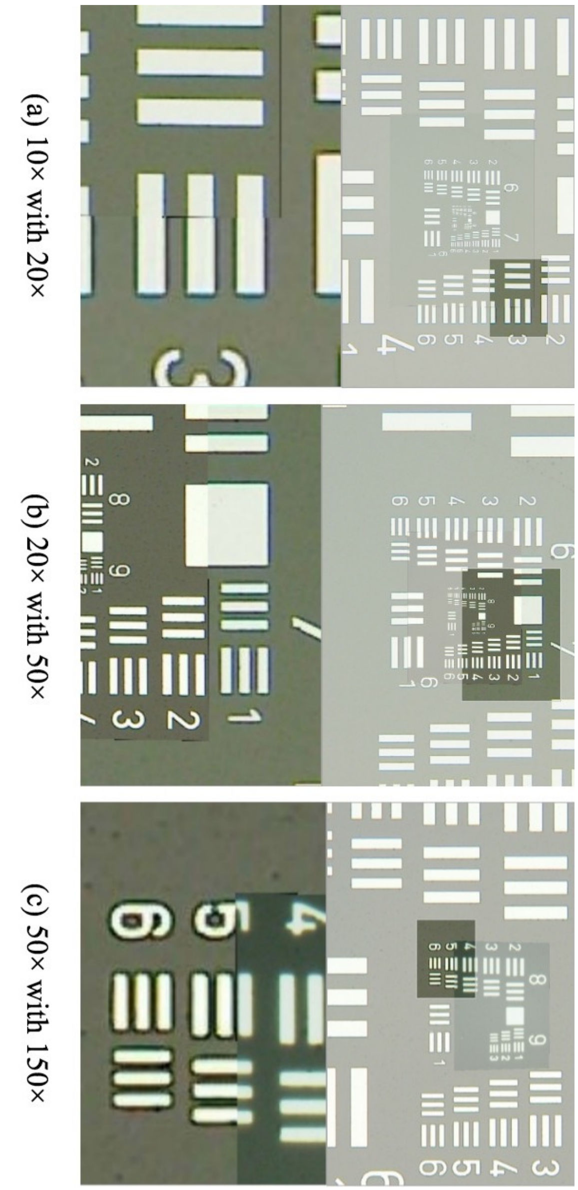

Figure 14: Three synthesis image type being magnified inside post-processing phase

Conclusion

Finally microscope image matching in scope of multiresolution image observation system has been done. By utilizing synthesis image to enhancing image crispness of high frequency image, data learning can be done smoothly by bag of words methods. The data learning model will be serialized and deserialized in order to make the prediction balance, neither underfitting nor overfitting. At the end, web application has been develop to view the image data from pre-processing including the result of recognition of the image. With this system, when user has to check and observe the bad part of the PCB board, cut circuit path, or looking for another small electronic components, this system can help to find the interest part that has been input by user.

We realize this work still has a drawback especially with the automation and the data learning. When the data become larger and vary, it would slow down the computation. And still this system still cannot differentiate between patch object image slightly similar like 6 with 9. Therefore, a future development of this work will be needed.

Future enhancement are needed to correct some of the drawback that happened such as virtual polysemy which leading to an ambiguity of the meaning behind the vocabulary. Beside of that, an idea of multispectral [6] image processing will gave a new level of observation system. As we can see Kleber et al use a several spectral band LED from infrared (IR) at peak wavelength 940 nm, 535 nm, 505 nm, 450 nm, 365 nm, and the last is visible light which build RGB image. They purpose of this work is to compare which light source give the best contrast image compare with the RGB image took by visible light. Multispectral image intend to enhance contrast of the images and thats make an observation become more accurate.

Another project which can enriched our project in the future is utilize a multispectral data for classification using SVM[1]. Bahari et al try classify area coverage on Klang valley in every pixel in the land area. Their work also measure accuracy assessment by using Kappa coefficient value. The result is their accuracy of kappa coefficient is 97.1%and that indicating reliability and compromising sensing classification tool.

Acknowledgments

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