美和學校財團法人美和科技大學

105 年度教師產學合作計畫
結案報告書

計畫名稱：智慧型可攜式微流體紙晶片影像檢測演算法與裝置之研發

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The Development of Image Base, Portable Microfluidic Paper-Based Analytical Device

摘要：本研究主要針對微流體紙晶片影像檢測演算法來進行影像分析與比較，並設計出適合的影像處理演算法，微流體紙晶片可用於特定疾病的初步篩檢，具有成本低與檢驗速度快的優點，使用微流體紙晶片需將特定檢測液滴在紙晶片的流道上，檢測液經由流道流至檢測區進行化學反應，反應後的紙晶片會呈現顏色的變化，只要依據顏色變化程度，即可找出相對應的疾病程度。先前文獻研究所使用影像檢測方法存在著許多問題，無法檢測出不準確的結果；為此，先前研究已針對微流體紙晶片的檢驗需求，開發一以影像處理為基礎之可攜式微流體紙晶片檢測裝置，接下來將針對顏色檢測所需的影像處理演算法來進行色彩分析，以期能獲得最佳的影像檢測結果。在影像檢測的方法上，主要是以色彩量化為基礎的影像分析演算法配合自行設計的特徵，找出紙晶片反應後的顏色；本研究使用了一般常見之色彩平均法、均勻色彩量化法、Median-cut 演算法、K-Means 分群演算法、自我組織類神經網路演算法等色彩量化法來進行比較，在本文章的實驗結果中，將進行上述色彩量化方法的性能比較，同時驗證本研究所設計之影像演算法是可行，同時也能被實現在可攜式嵌入式系統裝置上。

***Abstract*—**Microfluidic paper-based analytical devices can be used in preliminary screening and diagnosis of specific diseases. It has the advantages of low costs and quick diagnosis. After chemical reaction, the paper chip will display the disease's relative status through color identification or electrochemistry testing. When compared to electrochemistry testing, the non-contact color identification method has the advantages of low costs, reusability and not requiring cleaning. However since research in the past have encountered many problems with the color identification method, this has led to the negation of the advantages of microfluidic paper-based analytical devices in quick testing. For this reason, this study developed a portable testing apparatus with image analysis as a foundation and uses a self-designed imaging processing calculation method to implement color identification. The hardware equipment designed for image processing in this study uses a micro embedded style system and imaging equipment as the hardware framework, along with a Web application to implement a multi-platform operating interface. Color quantization was used as the foundation for the image processing method, to calculate the paper chip's color. Furthermore, this study also carried out a variety of color quantization methods to compare the color quantization results of the microfluidic paper-based analytical devices. Methods include uniform color quantization, median-cut algorithm, k-means clustering algorithm and self-organizing maps neural networks. During the experiment phase of this study, we found that median-cut was superior to other methods in application. This result is different from many other research works conducted on color quantization. Lastly, the experiment results showed the usability of this testing setup.

Keywords—microfluidic paper-based analytical devices; digital image processing; color quantization; embedded system;

Introduction

The μPAD research is mainly conducted by the Whitesides research team. Martinez et al. [1] of the Whitesides team deployed artificial urine liquid following literature review. They used the home-designed μPADs for glucose and protein detection, used the camera or scanner in cellular phone for image capturing to obtain the images on paper-based chips, transformed the colors on paper chip into numerical signal using Photoshop software and manual selection of color area, and accessed the outcome using the Colorimetric Assays. This method has several limitations, as it does not take into account the differences in physical characteristics between imaging equipment, thus it is difficult to determine the test reaction area, and as a result, only the mean values of the colors can be used. Other important literature related to the μPADs development are listed as [2~7]. In Taiwan, Chen et al. [8] performed an experiment in which the protein value in simulated human urine was above the standard value; such experiments can be used to reflect the human kidney diseases. The test used bovine serum albumin as test specimens, which can interact with tetrabromophenol blue (TBPB). The reaction mechanism is based on the non-specific binding property of TBPB with proteins. TBPB binds to proteins through the combination of electrostatic (sulfonate) and hydrophobic (biaryl quinone methide) interactions. Upon binding, TBPB becomes deprotonated, and changes color from yellow to blue. By adjusting the bovine serum albumin concentration, the concentration-color relationship can be observed in the detection area. Because the test results must be detected immediately after the reaction of the detection liquid has occurred on μPADs, corresponding detection devices are needed according to the function of μPADs. Detection devices must be portable and easy to use, but the detection method cited by Martinez et al. in the literature [1] does not satisfy the criterion of being portable, and many human error-related problems were also present in their detection method for the μPADs reaction. The μPAD research has become important in recent years, and detection equipment, which determines the results of μPADs reaction, plays as important a role as the μPADs research. For this reason, in the present study, we collaborated with the Chen research team, which is devoted to the research and development of μPADs, to develop an image-processing-based portable μPADs detection device, we aim to resolve the current problems related to μPADs detection, develop a device that is portable and to make the detection process automatic.

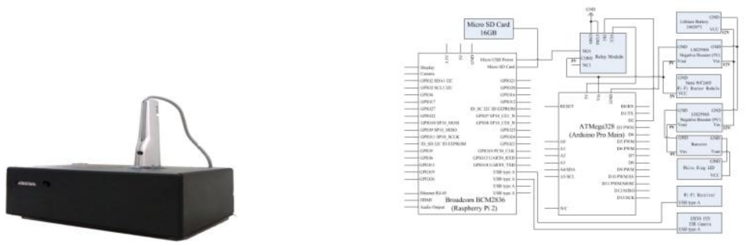
Regarding the device, the present study utilized the camera-based test as a tool, the low-cost system on chip (SoC) chips as the core element, the Embedded Linux as operating system which has low capacity, supports floating-point operations and provides multimedia services, in combination with ordinary home USB camera with LED lighting as image capturing components, as well as home-designed design device circuit and image processing algorithms to achieve the goal of developing a portable μPADs detection device. The detection device has three parts: a image-capturing unit (camera and light), an image analysis unit (image processing and result comparison), and a human-machine interface (operation interface and communications links). The consistency of image-capturing environment can ensure the image quality and increase the success rate, therefore we used a dark-colored plastic box as a small image-capturing chamber, take like room, use a LED white ring lamp equipped with Stabilizer as light source to avoid shadow or uneven lighting. The image analysis unit is designed according to the uneven color distribution in the μPADs reaction area. The color analysis method is based on the color quantitative concept, and can identify the representative color in the μPADs reaction area. For the purpose of finding out the optimal image detection method, we used a variety of color quantization methods for analysis in this study. We compared the use of the commonly used uniform color quantization[9], median-cut algorithm[10], k-means clustering algorithm[11], and self-organizing maps neural networks[12] along with other color quantization methods.

To reduce equipment cost and improve usability, this device is loaded with the Raspbian operating system and wireless AP, equipped with the Node.js Server services, uses HTML as operation interface, and the JavaScript programming language to implement image processing algorithms; as a result, through any device with a Web browser, users can link this device to the network and operate. Furthermore, efficiency of the browser used in the front-end and the SoC computation of the back-end proposed by this study will also be considered. Details of the methods will be explained in the section below.

Method

Device architecture

Regarding the construction of the device, the camera-based detection method is adopted to build a portable μPADs detection device (as shown in Figure 1), due to the advantages of paper chip being low cost and easy to carry, the switch of the detection device does not include the x86 hardware schema, instead the lower cost SoC chip being used as core element. This was because this device requires image capturing, algorithm calculation, and provides communications service, the Raspberry Pi 2 is used as the central control unit. This chip can be loaded with the Embedded Linux operating system Raspbian which has a small capacity and supports floating-point operations and provides multimedia services. This device uses the Raspberry Pi 2 in combination with the Raspbian OS as operating core to carry out μPADs response detection, the detection device has three functional parts: the imaging unit, the image analysis unit, and the human-machine interface unit.



(a) (b)

Fig. 1. (a)The developed portable μPADs detection device. (b) Circuit diagram.

Image Processing

Because in the μPADs production process wax splashing often occurs, prior to color quantization the noise (wax) image must be deducted before image processing to obtain noise-free images, this will ensure the color quantization results are not tainted by shades of flow channels and wax spots. Flow channel and wax noise on paper chip have obvious differences in color compared with backgrounds, the binary method can be used to remove most of the non-testing images from the testing zone, this threshold of binarization was obtained using the Otsu's threshold algorithm [13]; next, using the fast connected-component labeling algorithm [14] for area tagging, keeping only the central area for color quantization.

Color Quantization Methods

Color quantization was used in conjunction with pixel filtering to determine the representative color of the detection fluid. This method results in more accurate results than using the color averaging method used in previous studies. There is no way to accurately evaluate the main color from the reactive area using the color averaging method, but by using the color quantization method, choosing the reactive area and the main color evaluation problem can be avoided. Color quantization is mainly divided into two categories: clustering and segmented calculation methods. The advantages of clustering is that it is able to obtain a relatively optimal and precise quantization results but it also takes a longer computation time comparatively. At the same time, algorithm convergence and the problem of calculation speed need to be considered. Segmented calculation turns out to be the opposite, it has fast calculation speed but the quantization quality is inferior to clustering calculations. For the purpose of finding the most applicable method, we carry out comparisons of the results from various methods for color evaluation.

- "Uniform" quantization [9] is a commonly seen and used method for image color quantization. The advantage of this method is that processing is fast. This method is used within the API function, but the quantization results are not objective. Therefore it is not suitable for use in applications that require high precision in color evaluation.
- "Median-cut" algorithm was proposed by Heckbert in 1982 [10] and is a type of non-average color quantization method. This method uses analysis techniques to segment the color space repeatedly until a similar color is reached. The surface is segmented vertically with a single color axis, and the element with the largest pixel difference range out of the three color axis will be used as a standard for segmenting. Sorting is used and then the middle gray scale value is used as the segmenting point.
- "K-means Clustering" algorithm (KM) is a clustering algorithm proposed by MacQueen in 1967. The amount of clusters must be defined before using the algorithm. Afterwards the center point of the cluster, the sum of the distance between the vector points and the extreme values need to be found to achieve the goal of optimal clustering. In 1995 Verevka applied this method to color quantization[11].
- "Self-organizing Maps" (SOM) neural networks, in 1994 Dekker proposed a color quantization method using SOM[12]. It has relatively quick calculation time, and is able to raise sample counts and significantly improve quantization quality. This method mainly employs a one dimensional self-organizing map where the network contains every cluster's neurons. Through a self-learning process, every neuron obtains a weight vector which has representative value. After self-learning, the pixels are also reflected in the nearest weight vector.

Representative Color

Due to the fact that after color quantization, the coloring is not only of one single color, in order to find the main color, this method takes the color with the most pixels to be the representative color for the examined section. This method complies with the method recognized by testing personnel.

Experimental Results

In this study, 5 experiments were carried out to evaluate the performance of the four types of color quantization methods and a self-designed calculation method. The first experiment uses a sample image with high texture variation with quantization set as 256 and 6 quantization colors to carry out RMSE comparison. The second experiment uses the results of color quantization of an image with low texture variation to undergo RMSE comparison. The goal of these two experiments is to show the response of the four color quantization methods using the two types of images. Afterwards, we analyzed the repeatability of the color quantization results in the third experiment. A μPAD sample is used for color evaluation, and undergoes color quantization 3 times, using RMSE to evaluate the result's repeatability. The fourth experiment is to compare the performance of the tests. Besides comparing the similarity of standard colors with specific concentration, the calculation time required for the μPADs image processing is also considered. This experiment also evaluates the time required for the Web front-end and the SoC back-end platform. The front-end equipment consists a HTC_One E9+ smartphone, while the back-end equipment consists a Raspberry_Pi_II. The last experiment is targeted at the representative color characteristics proposed by this study to find the most suitable color quantization amount. The following are the results for the various experiments:

Experiment 1: Comparison of the RMSE Results for Color Quantization of Images with High Texture Variation

This experiment used a 24-bit image for the 4 types of quantization methods to be applied. Six color quantization was used in this experiment and the results were compared using RMSE with the original image. The 6 color quantization results are shown in Table 1. The results from the 6 color quantization clearly show that the Uniform quantization method seriously lacks fidelity with a RMSE value as high as 60.97. This also indicates that when quantizing a small amount of colors, the results from methods that segment colors evenly are easily distorted. Median-cut and SOM quantization give similar results while KM method's RMSE is the lowest.

Experiment 2: Comparison of the RMSE Results for Color Quantization of Images with Low Texture Variation

This experiment used a 24-bit 320x240 pixel color gradient image for quantization result evaluation. Compared to the image in experiment 1 this image has lower texture variation. It will be easy to see if the quantized image exhibits a comparable gradient effect of the original image. This experiment will also use the 4 quantization methods to carry out 6 color quantization RMSE evaluations. The quantization results are listed in Table 2.

In the results for the 6 color quantization for images with low texture variation (Table 2), we can see that the Uniform method is unable to deal with images with a small amount of colors while KM and SOM results are also not ideal and are unable to recreate the gradient effect of the original image. This is because the initial weight vectors of KM and SOM determine the results The Median-cut method is able to produce reasonable quantization results, while its RMSE value is also the best among the four methods.

Experiment 3: Comparison for µPADs Sample Color Quantization Repeatability

The results for the tests of the same reagent should be consistent to satisfy the testing requirements, and to give the test personnel the required objective results. Hence, we carried out the repeatability experiment for µPADs image sampling. This experiment is targeted at the testing region after background processing for color quantization (in the image in Table 3, the background is black and will not undergo color quantization). The image dimension is 480x380. Because of the undesirable quantization results from the Uniform method, the experiment will only use Median-cut, KM and SOM methods for comparison. These three methods are also commonly compared in many other studies. The experiment results are shown in Table 3, with 6 color quantization. Due to the calculation's characteristics, after repeating the experiment 3 times, the Median-cut quantization results are the same while KM and SOM quantization results showed variations. In practice it seems that the repeatability of SOM is the worst with the highest variations followed by KM. In this experiment, the RMSE value for SOM was the highest while Median-cut had the best performance.

Experiment 4: Comparison of µPADs Sample Imaging Testing Performance

This experiment used the representative color obtained through the methods from the study and compared them with manually selected standard colors. This is then quantified using Euclidean distance. Furthermore, this experiment also carried out analysis for calculation time. This part of the experiment includes the front-end and back-end calculation times. The front-end calculation refers to using the Chrome browser on a smart phone and back-end calculation refers to calculations by the SoC on the Node.js platform. The calculation time is measured in units of milliseconds, and the experiment includes 5 experiment sample images and the results are shown in Table 4. From the similarities shown in Table 4, using Median-cut as the foundation quantization method, results in the optimal similarity with the original image from the 4 samples. Another sample obtained the best similarity with SOM. The back-end required 4 seconds to carry out calculations which is acceptable and the front-end browser only took 1 second. This is because the smart phone's processor is faster than the Rasberry pi 2's. Calculation time: Uniform<SOM<Median-cut<KM, the calculation time required for SOM is indeed better than median-cut and KM as indicated by Dekker.

Experiment 5: Analysis for the Most Suitable Median-cut Color Quantization Amount

After realizing that the Median-cut color quantization method is suitable for our application, this experiment is used to target the representative color characteristics proposed by this study, to find out the most suitable color quantization amount. Through analysis of 30 sample images, with a resolution of 480x380 pixels, 27 of the samples showed no significant variance in their RMSE value after setting the color quantization amount to 16. Hence 16 colors gives the best results for Median-cut. Due to the constraints of space, we have only listed the results for one sample on Table 5.

CONCLUSIONS

This study developed a portable testing apparatus with image analysis as a foundation and uses a self-designed imaging processing calculation method to implement color identification and found a suitable image processing method. In terms of image processing, besides pre-processing and representative color characteristics, color quantization quality determines the test results. This study compared 4 quantization methods and found through experiment that the quantization quality of Uniform is unacceptable; Uniform is the method used by previous studies. Furthermore, the three non-averaging quantization methods that are frequently discussed, obtained different results in our experiments when compared to studies in the past (many studies indicate that the Median-cut method is inferior to KM and SOM). We verified that KM and SOM is superior to Median-cut when processing quantization of images with rich texture variations, but it is a different story when dealing with images with low texture variations. Furthermore, in terms of application, Median-cut's characteristics are also superior to KM and SOM in quantization result repeatability. Because of the aforementioned results, Median-cut was chosen as the color quantization method in the setup. In future studies, we will use the results obtained from this study to further apply to other detection fluid testing, and continue to verify the applicability of this setup.

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TABLE I. COMPARISON OF THE RMSE RESULTS FOR COLOR QUANTIZATION OF IMAGES WITH HIGH TEXTURE VARIATION (6 COLORS)

Image	Uniform	Median-cut	KM	SOM
				
	RMSE:60.97	RMSE:25.99	RMSE:21.33	RMSE:25.71

TABLE II. COMPARISON OF THE RMSE RESULTS FOR COLOR QUANTIZATION OF IMAGES WITH LOW TEXTURE VARIATION (6 COLORS)

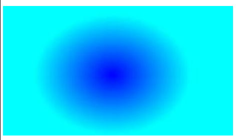
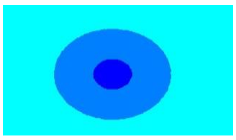
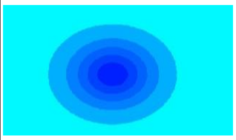
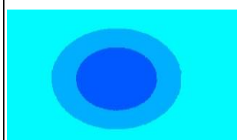
Image	Uniform	Median-cut	KM	SOM
				
	RMSE:15.63	RMSE:10.20	RMSE:12.67	RMSE:10.54

TABLE III. COMPARISON FOR μPADS SAMPLE COLOR QUANTIZATION REPEATABILITY (6 COLORS)

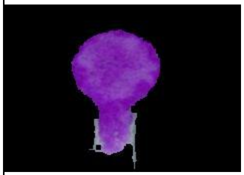
μ PADs (Original Image)		1st	2nd	3rd
	Median-cut			
		RMSE: 11.21	RMSE: 11.21	RMSE: 11.21
	KM			
		RMSE: 19.20	RMSE: 15.34	RMSE: 19.38
	SOM			
		RMSE: 38.27	RMSE: 29.27	RMSE: 23.06

TABLE IV. COMPARISON FOR MPADS SAMPLE COLOR QUANTIZATION REPEATABILITY (6 COLORS/ SRGB:RGB OF THE STANDARD SAMPLE COLOR)

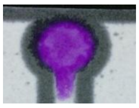
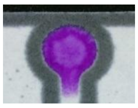
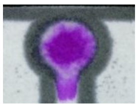
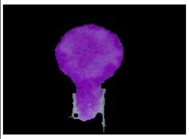
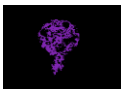
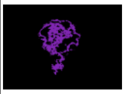
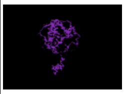
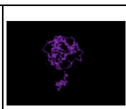
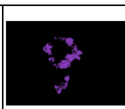
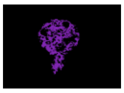
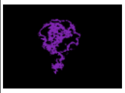
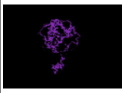
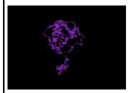
Samples		Uniform	Median-cut	KM	SOM
	sRGB:(123,25,170)	back-end:245	back-end:2821	back-end:3210	back-end:2277
		front-end:55	front-end:461	front-end:904	front-end:114
		similarity:49.13	similarity:3.60	similarity:4.58	similarity:10.48
	sRGB:(123,25,170)	back-end:246	back-end:2861	back-end:3490	back-end:2220
		front-end:50	front-end:487	front-end:928	front-end:121
		similarity:49.13	similarity:5.19	similarity:40.60	similarity:7.87
	sRGB:(116,12,150)	back-end:241	back-end:2964	back-end:4353	back-end:2203
		front-end:54	front-end:465	front-end:667	front-end:161
		similarity:27.78	similarity:11.70	similarity:14.00	similarity:39.50

TABLE V. ANALYSIS FOR THE MOST SUITABLE MEDIAN-CUT COLOR QUANTIZATION AMOUNT (CN: COLOR NUMBER)

Sample	CN: 4	CN:6	CN:8	CN:10	CN:12

					
	RMSE: 11.46	RMSE:10.42	RMSE:7.85	RMSE:7.85	RMSE:9.57
	CN:14	CN:16	CN:18	CN:20	
					
	RMSE: 9.44	RMSE: 5.72	RMSE: 5.72	RMSE: 5.72	

REFERENCES

[1] A.W. Martinez, S.T. Phillips, E. Carrilho, S.W. Thomas, H. Sindi, and G.M. Whitesides, “Simple Telemedicine for Developing Regions: Camera Phones and Paper-Based Microfluidic Devices for Real-Time, Off-Site Diagnosis,” Analytical Chemistry, vol. 80, pp. 3699-3707, 2008.

[2] Z. Chen, X. Liu, “A Paper-Based Microfluidic Device for Multiplexed Electrochemical Detection of Biomarkers,” 2013 International Conference on Manipulation, Manufacturing and Measurement on the Nanoscale (3M-NANO), pp. 162-165, 2013.

[3] X.Y. Liu, C.M. Cheng, A.W. Martinez, K.A. Mirica, X.J. Li, S.T. Phillips, M. Mascareñas, and G.M. Whitesides, “A Portable Microfluidic Paper-Based Device for ELISA,” 2011 IEEE 24th International Conference on Micro Electro Mechanical Systems (MEMS), pp. 75-78, 2011.

[4] X. Li, Z. Chen, and X. Liu, “An Electrochemical Microfluidic Paper-Based Glucose Sensor Integrating Zinc Oxide Nanowires,” 2015 28th IEEE International Conference on Micro Electro Mechanical Systems (MEMS), pp. 447-450, 2015.

[5] Y. Fan, H. Li, Y. Yi, and I.G. Foulds, “Low-Cost Rapid Prototyping of Flexible Plastic Paper Based Microfluidic Devices,” 2013 8th IEEE International Conference on Nano/Micro Engineered and Molecular Systems (NEMS), pp. 175-178, 2013.

[6] C. Hong, M. Faghri, C. Anagnostopoulos, “Paper- Based Non-Mechanical Valves for Autonomous Multi-Reagent Lateral Flow Microfluidic Devices,” 2014 IEEE Healthcare Innovation Conference (HIC), pp. 304-306, 2014.

[7] S.-I. Yang, K.F. Lei, S.-W. Tsai, and H.-T. Hsu, “Development of A Paper-Based Carbon Nanotube Sensing Microfluidic Device for Biological Detection,” 2013 35th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), pp. 168-171, 2013.

[8] J.-J. Chen, X.-C. Qiu, C.-M. Shen, S.-J. Chen, Y.-W. Ko, “A Microfluidic Device Integrated Active Cooling Channel for DNA Amplification,” Transactions of the Canadian Society for Mechanical Engineering, vol. 37, No. 3, pp. 655-664, 2013.

[9] R.C. Gonzalez, R.E. Woods, Digital Image Processing, 2nd ed., Prentice-Hall, 2002.

[10] P.S. Heckbert, , “Color Image Quantization For Frame Buffer Display,” Comput Graphics, vol. 16, No.3 , pp. 297-307, 1982.

[11] O. Verevka, “Color Image Quantization in Window System with Local K-means Algorithm,” Proc. Western Computer Graphics Symposium, pp. 74-79, 1995.

[12] A.H. Dekker, “Kohonen Neural Networks for Optimal Colour Quantization,” Network: Computation in Neural Systems, vol. 5, pp. 351-367, 1994.

[13] N. Otsu, “A Threshold Selection Method From Gray Level Histogram,” IEEE Transaction on Systems, Man, and Cybernetics, vol. 9(1), pp. 62-66, 1978.
[14] L.H.Y. Chao, K. Suzuki, and K. Wu, “Fast Connected-Component Labeling,” Pattern Recognition, vol. 42, pp. 1977-1987, 2009