Microarray Image Processing Based on Clustering and Morphological Analysis

Shuanhu Wu[1] and Hong Yan[1,2]

[1]Department of Computer Engineering and Information Technology
City University of Hong Kong, Kowloon, Hong Kong, Tat Chee Avenue, Kowloon, HongKong
[2]School of Electrical and Information Engineering
University of Sydney, NSW 2006, Australia Australia

E-mail: itwush@cityu.edu.hk and h.yan@cityu.edu.hk

Abstract

Microarrays allow the monitoring of expressions for tens of thousands of genes simultaneously. Image analysis is an important aspect for microarray experiments that can affect subsequent analysis such as identification of differentially expressed genes. Image processing for microarray images includes three tasks: spot gridding, segmentation and information extraction. In this paper, we address the segmentation and information extraction problems, and proposed a new segmentation method based on K-means clustering and a new background and foreground correction algorithm based on mathematical morphological and histogram analysis for information extraction. The advantage of our method is that it does not have any restrictions for the shape of spots. We compare our experimental results with those obtained from the popular software GenePix.

Keywords: DNA gene expressions, DNA chip data processing, Microarray image segmentation, K-means clustering, Mathematical morphology.

1 Introduction

DNA microarrays allow monitoring of expressions from tens of thousands of genes simultaneously. This technology (Schena, Shalon, et al. 1995; Schena, et.al. 1998) makes use of the so-called hybridization reaction that two lengths of single-stranded DNA will bind together or hybridize only if the bases on one strand find complementary bases on the other strand. So it makes possible to quantitatively analyze fluorescence signals that represent the relative abundance of mRNA from two distinct samples. Applications of microarrays range from the study of gene expression in yeast under different environmental stress conditions to the comparison of gene expression profiles of tumors from cancer patients. In addition to the enormous scientific potential of DNA microarrays to help in understanding gene regulation and interactions, microarrays have very important applications in pharmaceutical and clinical research. By comparing gene expression in normal and abnormal cells, microarrays may be used to identify genes that are involved in particular diseases.

Image processing is an important part for extracting and quantitatively analyzing the relative abundance of mRNA on the microarray images since it affect the following step of gene expression data clustering and analysis. In a microarray experiment, the hybridized arrays are imaged to measure the red (Cy5) and green fluorescence (Cy3) intensities for each spot on the glass slide. The processing of scanned microarray images includes three main tasks: spot gridding, segmentation and intensity extraction. Gridding is the process of assigning coordinates to each of the spots. Segmentation allows the classification of pixels either as foreground that is corresponding to a spot of interest, or as background. The intensity extraction includes calculating red and green foreground fluorescence intensity pairs (R, G), and background intensities.

In the last several years, a number of image processing technologies have been proposed for microarray images and several commercial software and freeware packages, such as ScanAlyze (Eisen 1999), GenePix (Axon Instruments, Inc. 1999), Spot (Buckly 2000) and QuantArray (GSI Lumonics 1999), have become available. Gridding can be obtained by automatic or semiautomatic means according to the configuration of microarrays. In this paper, we address two other problems: segmentation and intensity extraction. Existing segmentation methods for microarray images can be categorized into four groups according to geometry of the spots they produce: fixed circle segmentation, adaptive circle segmentation, adaptive shape segmentation and histogram based segmentation. Fixed circle segmentation fits a circle with a constant diameter to all the spots in the image. The advantage of this method is its easy implementation and works correctly when all the spots are circular and of the same size (Eisen 1999). The adaptive circle segmentation is adopted by GenePix (Axon Instruments, Inc. 1999). The circle's diameter is estimated separately for each spot. In practice, however, spots are rarely perfectly circular and can exhibit oval or donut shapes (Eisen and Brown 1999), thus segmentation algorithms that do not place restrictions on the shape of the spots are desirable. Two commonly used methods for adaptive segmentation in image analysis are the watershed (Beucher 1993, Vincent L 1991) and seeded region growing (SRG) (Adams 1994). The segmentation method implemented in the software package Spot is SRG. The weakness of both watershed and SRG segmentation methods is that they require the specification of starting points, or seeds. Histogram based segmentation method uses a target mask, which is chosen to be larger than any spot. For each spot, foreground and background intensity estimates are determined in some fashion from the histogram of pixel values for pixels within the masked area. The segmentation method adopted by Chen et al. (1997) uses a circular mask and computes a threshold value based on a Mann-Whitney test. In addition to segmentation method, the extraction method of information extraction for spot intensity also is a very important step. The natural measure of spot intensity is taking the median or average value of pixel intensities within the spot mask. Since there are some “noises” or inappropriate segmentation that may result in incorrectly evaluating background or foreground intensity due to inappropriate segmentation, some corrections may be needed for segmentation results. Software packages Spot (Buckly 2000), ScanAlyze (Eisen 1999) and GenePix (Axon Instruments, Inc. 1999) proposed a method for intensity extraction of background, such as median from “valley of spot” and median value in local square region, to correct the results of segmentation.

In this paper, we propose an adaptive shape segmentation method based on K-means clustering algorithm. In addition, we also proposed a background and foreground correction methods for the results of segmentation based on mathematical morphology and histogram statistics. This is important for evaluating the background and foreground intensities correctly. The advantages of our segmentation method are that there are no spot shape restrictions and that no threshold, or seed point is needed. In addition, our background correction method can also be used in combination with exiting software packages described above.

The paper is organized as follows. Section 2 introduces our K-means based segmentation method. In Section 3, we present a new foreground and background correction method for information extraction. The experimental results and comparisons are given in Section 4 and conclusions are drawn in Section 5.

2 K-means based segmentation method

2.1 Preprocessing

We represent input images by “R” and “G” for “red” and “green” with R corresponding to the Cy5 and G to Cy3 respectively. Since the gridding and segmentation stages require a single image, raw images R and G must be combined into one that should not be dominated by either of the two inputs. In addition, it is also convenient computationally for the combined image to be an 8-bit image, so the automatic addressing and segmentation procedures can be performed on the 8-bit combined image. The segmentation method will produce a spot mask that is used together with the original 16-bit images for extraction of spot foreground and background intensities.

Let $\{R(i, j\} \mid i = 1,2,...M; j = 1,2,...N\}$ be the image corresponding to the Cy5 and $\{G(i, j\} \mid i = 1,2,...M; j = 1,2,...N\}$ to Cy3. The following procedures are used to produce an 8-bit combined image, $\{RG(i, j\} \mid i = 1,2,...M; j = 1,2,...N\}$: (1) First, we can obtain two combined 8-bit image, R' and G' by a square-root transformation that is applied to both the inputs, R and G, respectively. This also can reduce the domination of very bright pixels in the segmentation stage; (2) Then a combined 8-bit image can be formed according to following formula:

$$RG(i, j) = \max\{R'(i, j), G'(i, j)\}.$$

In the final, we adopt a similar automatic algorithm proposed by J. R. Hirata in (Hirata 2001) for addressing or gridding the combined 8-bit image RG.

2.2 Spot segmentation based on K-means clustering method

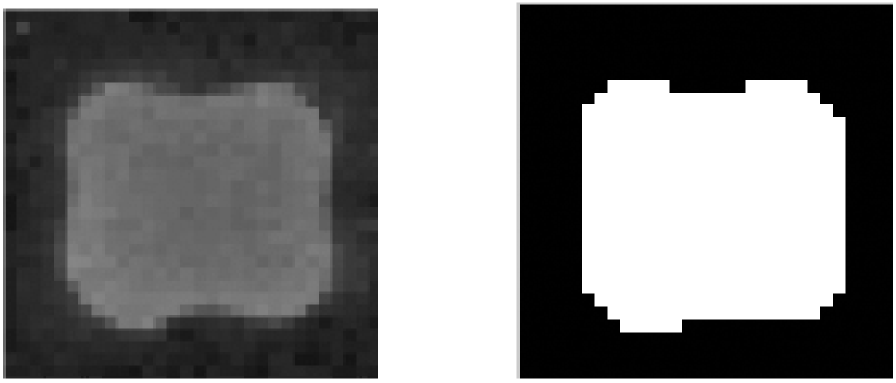
Figure 1(a) is one of gridded spot images from above combined 8-bit image, RG. Our objective is to segment it for extracting spot foreground and background intensities in the next stage without any shape restrictions for the objects that will be processed. K-means clustering algorithm is the one that is well suitable for our problem. Generally, K-means algorithm (Hartigan 1975) is an iterative clustering algorithm, so it seems to be very slow. However, if we initialize it with appropriate partitions in the beginning of iteration, it can be quite fast. In addition, our objective is only to segment spot image into two categories, foreground and background, so the algorithm can be implemented efficiently.

Let $\{Spot(i, j\} \mid i = 1,2,...m; j = 1,2,...n\} \subset RG$ be the spot image that will be segmented. We use $\{SpotType(i, j\} \mid i = 1,2,...m; j = 1,2,...n\}$ to indicate the type of the cluster that a pixel in *Spot* belongs to and the definition of $SpotType(i, j\}$ is as follows:

$$SpotType(i, j) = \begin{cases} 0, & background\ \ pixel \\ 1, & foreground\ \ pixel \\ 2, & noise \end{cases} \qquad (1)$$
$$i = 1,2,...m; \quad j = 1,2,...n$$

The type of $SpotType(i, j\} = 2$ will be used for the foreground and background corrections in next section. The center of two clusters that represent background and foreground is the mean of intensities that belongs to background and foreground, respectively. We denote *MeanBack* and *MeanFore* as background and foreground, the numbers of the pixels *NumberBack* and *NumberFore* for background and foreground, respectively. Our segmentation algorithm based K-means clustering can be described as follows:

Step 1: Initial clustering. Let *MinPixel* and *MaxPixel* be t he minimum and maximum value of intensity in the spot t hat will be segmented and *NumberFore*=0, *NumberBack*= 0. If |*Spot*(*i, j*) - *MinPixel*| > |*Spot*(*i, j*) - *MaxPixel*|, Then *S pot*(*i, j*) belongs to foreground, let *SpotType*(*i, j*) = 1, *Num berFore* = *NumberFore* + 1; Otherwise, it belongs to back ground and let *SpotType*(*i, j*) = 0, *Numberback* = *number-back* + 1. After this process, we can calculate the center



(a) Original spot image (b) Image after segmention

Figure 1: The result of segmentation by the K-means clustering algorithm

as follows:

$$MeanFore = \sum_{SpotType(i,j)=1} Spot(i,j) / NumberFore$$
$$MeanBack = \sum_{SpotType(i,j)=0} Spot(i,j) / NumberBack \quad (2)$$

Step 2: For the *Spot*(*i, j*) that *SpotType*(*i, j*) = 0, if

$$\frac{NumberBack * |Spot(i,j) - MeanBack|}{NumberBack - 1} > \frac{NumberFore * |Spot(i,j) - MeanFore|}{NumberFore + 1} \quad (3)$$

Then move $Spot(i, j)$ from the background cluster to the foreground cluster, i.e. let *SpotType*(*i, j*) = 1, *NumberFore* = *NumberFore* + 1 and *Numberback* = *Numberback* –1 and adjust the cluster means of two clusters according to (2). The similar calculations can be applied to the *Spot*(*i, j*) for which *SpotType*(*i, j*) = 1.

Step 3: If there is no movement between two clusters representing background and foreground, stop. Otherwise, repeat Step 2.

Initial clustering in Step 1 is very efficient for speeding up the convergence of K-means algorithm. In general, it can finish the process of segmentation with only one or two iterations. Figure 1 is the results of segmentation using above algorithm. Figure 1(a) is the original spot image. Figure 1(b) is the binary image representing the image *SpotType* after segmenting. White area and black area represent foreground and background, respectively.

3 Segmentation corrections and information extraction

The objective of spot image segmentation is to extract the intensity information. A common method for extracting the foreground or background intensities is to calculate their mean or median, respectively. Since noise can cause inappropriate segmentation, corrections of the results are needed. In this section, we first propose a correction method based on mathematical morphology and histogram statistics.

3.1 Foreground corrections based on mathematical morphology

Figure 2(a) is a binary image, from the result of segmenting of one spot. It is apparent that some pixels in the background area do not belong to the foreground and in the meanwhile, also do not belong to background in the stage of information extraction, so we regard those pixels as noise and need to exclude it. Mathematical morphology is a powerful tool for geometrical shape analysis and is suitable for solving this problem. We briefly review some morphological operations relevant to our algorithm below. More detailed discussions of mathematical morphology can be found in the literature written by Vincent (1993) and Haralick (1992).

A binary image can be considered as a subset of $E \times E$, where E denotes the set numbers used to index a row and a column position on a binary image. Pixels are in this subset if and only if they have the binary value “1” on the image. The dilation of set $A \subset E^2$ with structuring element $B \subset E^2$ is defined as follows:

$$A \oplus B = \bigcup_{b \in B} A_b \quad (4)$$

where A_b denotes the translation of A by a vector *b*, $\bigcup$ the pixel-wise union. The erosion of set $A \subset E^2$ with set $B \subset E^2$ is defined as follows:

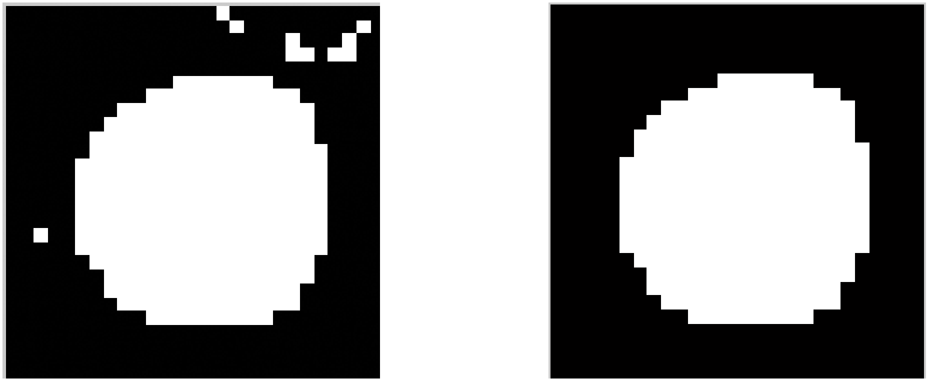
$$A \Theta B = \bigcap_{b \in B} A_b \quad (5)$$

where $\bigcap$ denotes the pixel-wise product. The dilation operation produces an enlarged set containing the original set A and some neighboring pixels. The erosion operations produce a skeleton image.

Let $A \subseteq E^2$ denote the set to be reconstructed and the marker $M \subseteq A$ that the value of pixels is “1” be an arbitrary subset of A. Then the conditioned dilation operation is defined as follows (Vincent 1993):

$$D^1(M, A) = (M \oplus B) \cap A \quad (6)$$

The succession of n conditioned dilation is defined as:



(a) Initial segmentation spot image (b) Spot image after correction

Figure 2: The result of foreground correction by mathematical morphology

$$D^n(M, A) = D^1(D^{n-1}(M, A), A) \tag{7}$$

To remove noisy points in the background such as those in Figure 2(a), we can perform a succession of *n* erosions by (5) first for the binary image, $\{SpotType(i, j\} \mid i = 1,2,...m;\ j = 1,2,...n\}$, from the result of segmentation above. Then we perform a succession of n conditional dilations by (7) for the result of erosions obtained previously. In practical processing, we use the 3×3 symmetric structuring element and $n = 2$. Finally we denote the result of morphological transformation by the binary image, $\{ f_M(i, j) \mid i = 1,...m;\ j = 1,...n\}$ and let

$$\begin{aligned} &SpotType(i, j\} = 2 \\ &for \quad i, j \in \{\{k, l \mid SportType\,(k, l) = 1\} \\ &\qquad - \{k, l \mid f_M(k, l) = 1\}\} \end{aligned} \tag{8}$$

where the set $\{i, j \mid SpotType(i, j) = 2\}$ represents the set of the positions of noisy pixels that just be excluded. Figure 2(b) is the result of foreground corrections.

3.2 Histogram analysis for background corrections

Besides previous foreground correction procedure, we also need to remove noise in the background before information extraction. We give such an example in Figure 3. Figures 3(a) and (b) are an original spot image and its histogram, respectively. We can see a transition area from background to foreground. The histogram in Figure 3(b) from about gray-level 30 to 55 is mainly contributed from this transition area. Calculating the pixels of this transition area is not appropriate in the stage of intensities extractions, so we should try to exclude it. Figure 4(a) is an example of background image segmented from a spot image showed in Figure 3 (a), its histogram is given in Figure 4(b). It is obvious that some pixels in transition area are partitioned into background area in the stage of segmentation.

For our problem, it is appropriate to regard that the histogram of each spot image has a bimodal structure and its two local maxima approximately correspond to the means of background and foreground area, respectively. After segmenting each spot image into background and foreground, the histogram corresponding to each part becomes unimodal. We assume that the histogram of background image satisfy Gaussian distribution that has a symmetric structure. If we can get the gray level of the maximum of histogram representing the mean of image approximately and variance corresponding to background area, then we can evaluate the range of gray level of background pixels and thence exclude noisy pixels in background area according to the Gaussian distribution.

In the following, we propose a correction method for excluding those noisy pixels in the background area based on histogram analysis. Since the image histogram is often noisy, so we first use following smoothing filter to smooth it several times:

$$\begin{aligned} &hist(i) = (hist(i-1) + hist(i) + hist(i+1))/3 \\ &hist(-1) = hist(256) = 0\,; \qquad i = 0,2,...255 \end{aligned} \tag{9}$$

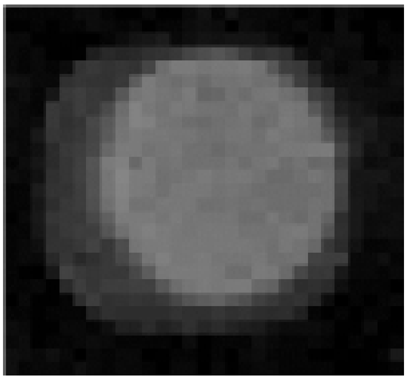
where $hist$ represents the histogram. Figure 5 (a) is the smoothing result of Figure 4 (b) of background area of one spot image. Since the mean approximately corresponding to the position of the maximum of histogram and can be easily obtained, so we only just need to evaluate the variance. Let *Mean_bg* be the mean that corresponds to the gray level of maximum of its histogram, and *Variance_bg* be the variance. *Variance_bg* can be evaluated by solving following equation according to the left half of histogram approximately:

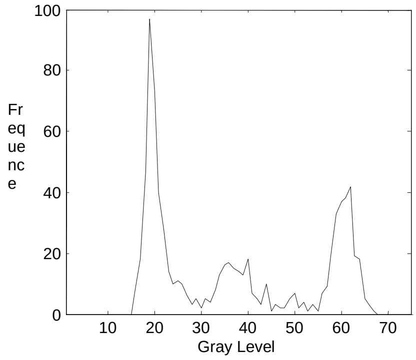
$$h(i) = a * \exp(-\frac{(i - Mean\_bg)^2}{2 * Variance\_bg}) \tag{10}$$

$$i \in \{k \mid hist(k) \neq 0 \ and\ k <= Mean\_bg\}$$

There are two parameters in above equation, *a* and *Variance_bg*, which can be obtained by data fitting. Let f_{back} be the set of pixels in background image that have not been corrected yet, then we can get approximately the set, denoted by $Back\_Pixels$, of noisy-free pixels according to Gaussian distribution as follows:

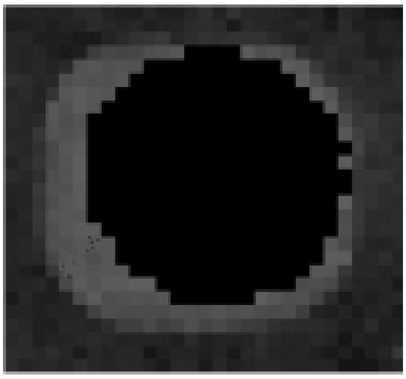
$$\begin{aligned} &Back\_Pixels = \{i, j \mid f_{back}(i, j) <= Mean\_bg \\ &+ 2 * Variance\_bg \ \text{ and } \ SpotType(i, j) = 0\} \end{aligned} \tag{11}$$

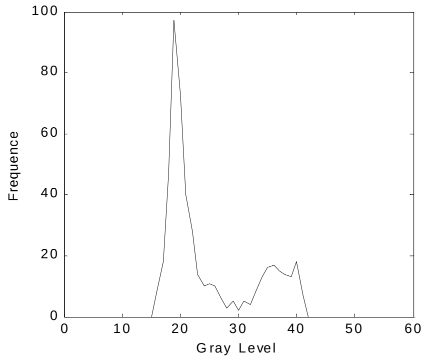




(a) Original spot image (b) The histogram of (a)

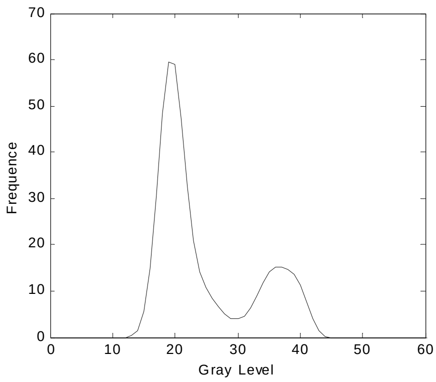
Figure 3: Original spot image and its histogram.

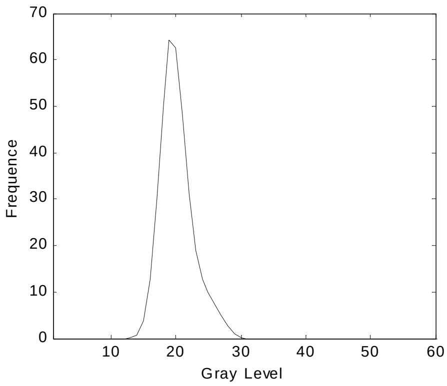




(a): Background image (b): The histogram of (c)

Figure 4: Background image and its histogram





(a) Smoothed histogram of background image (b) The histogram after background correction

Figure 5: The result of histogram correction.

And let

$$SpotType(i, j) = 2 \\ for \;\; i, j \in \{\{k, l \mid SpotType(k.l) = 0\} - Back\_Pixels\} \tag{12}$$

In Figure 5 (b), we show the histogram of background pixels that have been corrected. We can see that the noise part has now disappeared. Figure 6 is the noise part separated based on above algorithm.

In fact, the foreground part may also includes some noise due to inaccurate segmentation. It can also be corrected using a similar algorithm.

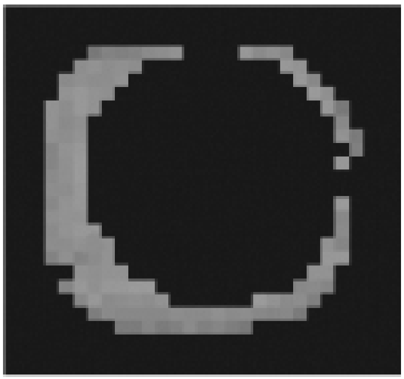


Figure 6: The noises image excluded from image in Figure 4(a)

3.3 Extraction of foreground and background intensities

After above image segmentation and correction procedures, we obtain three sets, $\{i, j \mid SpotType(i, j) = 0\}$, $\{i, j \mid SpotType(i, j) = 1\}$, $\{i, j \mid SpotType(i, j) = 2\}$, representing the background area, foreground area and noisy area, respectively. The procedures of intensity extraction for two 16-bit images, red image $\{R(i, j\} \mid i = 1,2,...M; j = 1,2,...N\}$ corresponding to the Cy5 and green image $\{G(i, j\} \mid i = 1,2,...M; j = 1,2,...N\}$ to Cy3, can be done based on the set of the pixels of corrected foreground and background areas.

Let $mean\_r\_back$, $median\_r\_back$, $mean\_r\_fore$ and $median\_r\_fore$ be the average and median value of the pixels in background and foreground area for one of red (Cy5) spot image, respectively, and $mean\_g\_back$, $median\_g\_back$, $mean\_g\_fore$ and $median\_g\_fore$ indicate the average and median value of the pixels in background and foreground area for one of green (Cy3) spot image, respectively. We can evaluate those values as follows:

$$mean\_r\_back = \sum_{SpotType(i,j)=0} R(i, j) / N_{back} \tag{13}$$

$$median\_r\_back = \underset{SpotType(i,j)=0}{Median}\{R(i, j)\} \tag{14}$$

$$mean\_r\_fore = \sum_{SpotType(i,j)=1} R(i, j) / N_{fore} \tag{15}$$

$$median\_r\_fore = \underset{SpotType(i,j)=1}{Median}\{R(i, j)\} \tag{16}$$

$$mean\_g\_back = \sum_{SpotType(i,j)=0} G(i, j) / N_{back} \tag{17}$$

$$median\_g\_back = \underset{SpotType(i,j)=0}{Median}\{G(i, j)\} \tag{18}$$

$$mean\_g\_fore = \sum_{SpotType(i,j)=1} G(i, j) N_{fore} \tag{19}$$

$$median\_g\_fore = \underset{SpotType(i,j)=1}{Median}\{G(i, j)\} \tag{20}$$

where N_{back} and N_{fore} are the numbers pixels in the set $\{i, j \mid SpotType(i, j) = 0\}$ representing corrected background area, and the set $\{i, j \mid SpotType(i, j) = 1\}$ representing corrected foreground area.

4 Experimental results

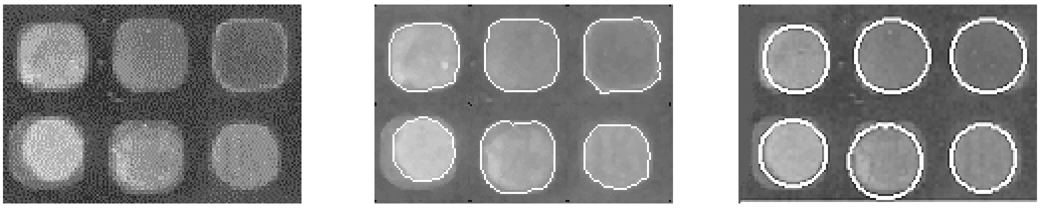
In this section, we present experimental results of spot image segmentation and intensity extractions using our algorithm described above. We compare our results with those obtained using the popular software package GenePix (Axon Instruments, Inc. 1999), which makes adaptive circle segmentation and estimates the circles diameter for each spot.

In Figure 7, we show the segmentation results by our algorithm and GenePix (Axon Instruments, Inc. 1999). Figure 7(a) is the original mini 2-by-3 spots image. Figure 7(b) is the spots image segmented by our algorithm and Figure 7(c) is the spots image segmented by Software GenePix. We can see that our segmentation algorithm has no shape restriction and the segmentation results fit the original spot shapes well. However, due to the restriction of using circular shapes, GenePix misclassifies some part of the foreground area as background or vice versa.

Finally, we test our intensity extraction algorithm for the spot image. Most microarray analysis packages define the foreground intensity as the median of pixel values within the segmented spot mask. More variation exists in the choice of background calculation method. Popular approaches do this by taking the median of values in selected regions surrounding the spot mask. The background intensities extracting method implemented by GenePix (Axon Instruments, Inc. 1999) is to calculate the median intensity or the mean from local valley regions. In general, GenePix adopt the median as a standard for foreground and background intensities extracting. Our background extraction method is based on histogram statistics described above and intensity calculation method can be based on either the median or mean. **Table 1** and **table 2** are the intensities extraction results for foreground and background, respectively, for the mini 2-by-3 spots image in Figure 7 by our method and GenePix. From the results of two tables, we can see that the intensities extracted by taking the median are comparable with the results by taking median by using GenePix. In addition, the results of intensity extracting are consistent for our algorithm whether taking the mean or the median. However, there are differences in the results of intensities for some spot, such as column 1 and row 1 in Figure 7(a), processed by GenePix. This may be caused from the inappropriate segmentation by circle. This shows that our algorithm is more accurate.

5 Conclusions

In this paper, we proposed an adaptive shape segmentation method based on K-means clustering and



(a) (b) (c)

Figure 7: Comparison of segmentation results. (a) shows the original spot image; (b) is the spot image segmented by our algorithm and (c) is the image segmented by GenePix.

Spot **R C**	**Median for Cy5 (Red)**		**Mean for Cy5 (Red)**		**Median for Cy3 (Green)**		**Mean for Cy3 (Green)**	
	Ours	**GenePix**	**Ours**	**GenePix**	**Ours**	**GenePix**	**Ours**	**GenePix**
1 1	3448	3558	3583	3818	2488	2597	2548	2738
1 2	1799	1809	1845	1878	1345	1372	1384	1412
1 3	1305	1244	1484	1299	935	901	1025	934
2 1	4713	4765	4512	4637	3534	3552	3371	3456
2 2	2681	2883	2706	2936	2041	2139	2037	2204
2 3	2687	2731	2681	2730	1817	1831	1817	1847

Table 1: Intensity extraction results for the mini 2-by-3 spots image from Cy5 (red) dye and Cy3 (green) dye shown in Figure 7(a). The table lists for each spot, the median and the mean of the spot *foreground* intensities estimated by our method and GenePix (Axon Instruments, Inc. 1999). The number below "R" in first column of table corresponding the row of the mini 2-by-3 spots image in Figure 7(a) and the number below "C" corresponding to the column.

Spot **R C**	**Median for Cy5 (Red)**		**Mean for Cy5 (Red)**		**Median for Cy3 (Green)**		**Mean for Cy3 (Green)**	
	Ours	**GenePix**	**Ours**	**GenePix**	**Ours**	**GenePix**	**Ours**	**GenePix**
1 1	534	521	563	599	373	352	387	408
1 2	474	489	492	523	324	333	344	365
1 3	535	501	560	555	355	348	371	388
2 1	509	498	519	590	342	335	352	403
2 2	486	492	493	532	347	342	356	381
2 3	565	518	583	578	391	369	419	422

Table 2: Intensity extraction results for the mini 2-by-3 spots image from Cy5 (red) dye and Cy3 (green) dye shown in Figure 7(a). The table lists for each spot, the median and the mean of the spot *background* intensities estimated by our method and GenePix (Axon Instruments, Inc. 1999). The number below "R" in first column of table corresponding the row of the mini 2-by-3 spots image in Figure 7(a) and the number below "C" corresponding to the column.

a background and foreground correction method for intensity evaluation based on mathematical morphology and histogram statistics. Since we adopted an efficient initial clustering procedure, the segmentation process based on the K-means algorithm is very fast and can be finished in one or two iterations. The advantages of our algorithm are that it has no shape restrictions and that there is no need for any thresholds or seeds. Experimental results show that our method is accurate and efficient. In addition, our image background and foreground correction methods can also be applied in combination with other segmentation algorithm.

Acknowledgement: This work is supported by CityU grants 7001183 and 9010003.

6 References

Adams, R., and Bischof, L. (1994): Seeded region growing. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, **16**(6): 641-547.

Axon Instruments, Inc. (1999): GenePix 4000A User's guide.

Beucher, S., and Meyer, F. (1993): The morphorlogical approach to segmentation: the watershed transformation. *In Mathematical morphology in image processing, volume 34 of Optical Engineering, chapter 12,* pages 433-481. Marcel Dekker, New York.

Buckly, M. J. (2000): The Spot user's guide. CSIRO Mathematical and Information Sciences. *http://www.cmis.csiro.au/IAP/Spot/spotmanual.htm.*

Chen, Y., Dougherty, E. R., and Bitter, M. L. (1997): Ratio-based decisions and the quantitative analysis of Cdna microarray images. *Journal of Biomedical Optics*, **2**(4): 364-374.

Eisen, M. B., and Brown, P. O. (1999): DNA arrays or analysis of gene expression. *Methods in Enzymology, 303*.

Eisen, M. B. (1999): ScanAlyze. *http://rana.stanford.edu/software for software and documentation.*

GSI Lumonics (1999): QuantArray Analysis Software, Operator's Manual.

Haralick, R. M. and Shapiro, L. G. (1992): Computer and Robot Vision. *Reading MA:* Addison-Wesley.

Haralick, R. M., Sternberg, S. R. and Zhuang, X. (1987): Image analysis using mathematical morphology. *IEEE Trans. Pattern Anal. Machine Intell.*, vol. **9**(7): 532–550.

Hartigan, J. A. (1975): Clustering algorithms. New York: Wiley Press.

Hirata, J. R., Barrera, J., Hashimoto, R. F., and Dantas, D. O. (2001): Microarray gridding by mathematical morphology. *Computer Graphics and Image Processing, 2001 Proceedings of XIV Brazilian Symposium on 2001*. Page(s): 112 –119.

Vincent, L. (1993): Morphological grayscale reconstruction in image analysis applications and effective algorithms. *IEEE Trans. Image Processing*, **2**(4): 176–201.

Vincent L., and Soille, P. (1991): Watersheds in digital spaces: An efficient algorithm based on immersion simulation. IEEE Transactions on Pattern Analysis and Machine Intelligence, **13**(6): 583-598.

Schena, M., Heller, R. A., Theriault, T., Konrad P., K., Lachenmeier, E., and Davis, R. W. (1998): Microarrays. Microarrays: biotechnology's discovery platform for functional genomics. *Trends in Biotechnology*, **16**(7): 301-306.

Schena, M., Shalon, D., Davis, R. W., and Brown, P. O. (1995): Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science*, **270**: 467-470.