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Automatic Gridding for DNA Microarray Image Using Image Projection Profile

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Abstract. DNA microarray is powerful tool and widely used in many areas. DNA microarray is produced from control and test tissue sample cDNAs, which are labeled with two different fluorescent dyes. After hybridization using a laser scanner, microarray images are obtained. Image analysis play an important role in extracting fluorescence intensity from microarray image. First step in microarray image analysis is addressing, that is finding areas in the image on which contain one spot using grid lines. This step can be done by either manually or automatically. In this paper we propose an efficient and simple automatic gridding for microarray image analysis using image projection profile, base on fact that microarray image has local minimum and maximum intensity at background and foreground areas respectively. Grid lines are obtained by finding local minimum of vertical and horizontal projection profile. This algorithm has been implemented in MATLAB and tested with several microarray images.

1 Introduction

DNA microarray is powerful tool and widely used in many areas, e.g. for human genetic research and drug discovery. DNA microarray is produced from control and test tissue sample cDNAs, which are labeled with two different fluorescent dyes, usually the red fluorescent dye Cy5 for the control and green fluorescent dye Cy3 for the test and then printed on a glass microslide containing gene specific cDNA clones arranged in an array format for hybridization. After hybridization using a laser scanner, images of the microarray are obtained [1].

Image processing and analysis play an important role in extracting fluorescence intensity from microarray image. there are The three basic steps of microarray analysis are:

1. Addressing: finding areas in the image on which contain one spot using grid lines. The combined area of the spot and its background is called the target area.
2. Segmentation: portioning the target area of every spots into two distinct regions, foreground (the spot its self) and background.

3. Reduction: extracting two scalar values for the intensity of R and G and assigning one value R/G for relative abundance to each spot.

The first step can be done by either manual grid alignment or fully automated (automatic gridding) [5].



Fig. 1. Microarray

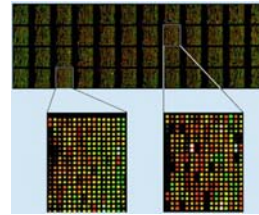


Fig. 2. Microarray image

Many available tools provide algorithms for microarray image analysis, among these, GenePix [3], Imagen [4], QuantArray [7] and ScanAlyze [6] are widely used. Most methods assume circular spot shapes and require manual alignment of the grid locations. Therefore, automated grid and spot finding are highly desirable. The Gridclus algorithm is an algorithm for microarray image automatic gridding based on k-mean clustering has been presented [5], but this algorithm is not efficient in time computing [12]. Another algorithm uses mathematical morphology, including image projection profile and other complex mathematical morphology operations [9]. In this paper we propose an efficient and simple automatic gridding for microarray image analysis using image projection profile, based on the fact that microarray image has local minimum and maximum intensity at background and foreground areas respectively.

2 Mathematical Backgrounds

In this section, we provide some mathematical backgrounds which are used in microarray image automatic gridding. Suppose \mathbb{Z} is the set of integers, E is a non-empty and finite subset of \mathbb{Z} and K is an interval $[0, k]$ of \mathbb{Z} , with $k > 0$. A function f from E to K , $f \in K^E$, represents a gray-scale image. A pixel is an element of E , $p \in E$ is a point in an image f and its gray-level is $f(p)$.

Definition 1. The union of two gray-scale images f_1 and f_2 , denoted by $f_1 \vee f_2$, is the function in K^E defined as, for any $p \in E$, $(f_1 \vee f_2)(p) = \max\{f_1(p), f_2(p)\}$.

Definition 2. For a pixel $(a, b) \in E$, $E_{x=a} \subset E$ is the cutting line E in vertical direction at (a, b) defined as $E_{x=a} = \{(a, y) \in E\}$. Similarly, $E_{y=b} \subset E$ is the cutting line E in horizontal direction at (a, b) defined as $E_{y=b} = \{(x, b) \in E\}$.

Definition 3. Suppose $f : E \rightarrow K$ is a gray-scale image, the horizontal projection profile of f , denoted by $P_h(f)$, is a function from $E_{x=0}$ to \mathbb{Z} defined as $P_h(f)(0, b) = \sum_{p \in E_{y=b}} f(p)$, for any $(0, b) \in E_{x=0}$. The vertical projection profile of f , denoted by $P_v(f)$, is a function from $E_{y=0}$ to \mathbb{Z} defined as $P_v(f)(a, 0) = \sum_{p \in E_{x=a}} f(p)$, for any $(a, 0) \in E_{y=0}$.

Definition 4. $P_h(f)$ has local maximum (resp. local minimum) at $(0, b_0)$ if $P_h(f)(0, b_0) \geq P_h(f)(0, b)$ (resp. $P_h(f)(0, b_0) \leq P_h(f)(0, b)$) for any $(0, b)$ in some neighborhood of $(0, b_0)$. $P_v(f)$ has local maximum (resp. local minimum) at $(a_0, 0)$ if $P_v(f)(a_0, 0) \geq P_v(f)(a, 0)$ (resp. $P_v(f)(a_0, 0) \leq P_v(f)(a, 0)$) for any $(a, 0)$ in some neighborhood of $(a_0, 0)$.

3 Automatic Gridding

Two matrix of intensity values are extracted from microarray image, one for the red channel and one for the green channel. Automatic gridding algorithm with projection profile is started with defining $R(i, j)$ and $G(i, j)$ as intensity values for the red and the green channel, for $i = 1, 2, \dots, M$, $j = 1, 2, \dots, N$, where M and N are the total number of pixel rows and columns respectively, on entire microarray image. The next steps for automatic gridding are:

1. Define f as union of R and G . the output is matrix \mathbf{f} .
2. Calculate horizontal projection profile $P_h(f)$ and vertical projection profile $P_v(f)$ for f . The output are vectors \mathbf{p}_h and \mathbf{p}_v .

3. Determine locations of local minimum for $P_h(f)$ and $P_v(f)$. The output are vectors \mathbf{m}_h and \mathbf{m}_v .
4. Determine locations of local maximum for $P_h(f)$ (resp. $P_v(f)$). To avoid local maximum in background area choose local maximum such that its value less than $\max\{\mathbf{p}_h\}/c$ (resp. $\max\{\mathbf{p}_v\}/c$) for some integer $c > 0$ (e.g. $c = 3$). The output are vectors \mathbf{M}_h and \mathbf{M}_v .
5. Between each pair of contiguous element of \mathbf{M}_h (resp. \mathbf{M}_v), determine smallest and largest elements of \mathbf{m}_h (resp. \mathbf{m}_v). Calculate average of such as smallest and largest elements of \mathbf{m}_h (resp. \mathbf{m}_v) as row (resp. column) grid lines.

This algorithm has been implemented in MATLAB and tested with several microarray images one of them is microarray image with resolution 531×533 pixels and contain 14×14 microarray spots as shown in figure 3. Figure 4 show union of figure 3, figure 5 and 6 show horizontal and vertical projection profiles of image shown in figure 4.

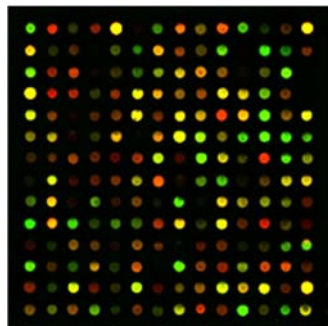


Fig. 3. Microarray image used in implementation

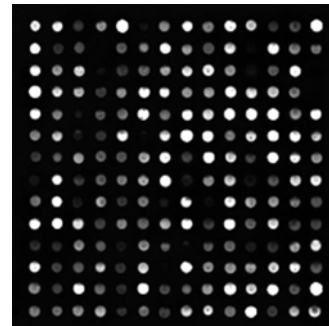


Fig. 4. Union of R and G

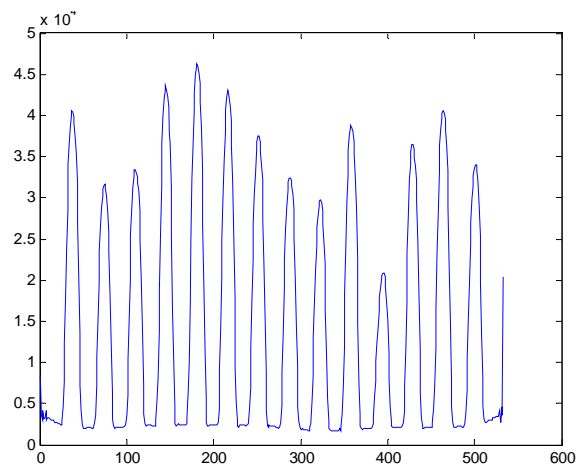


Fig. 5. Horizontal projection profile

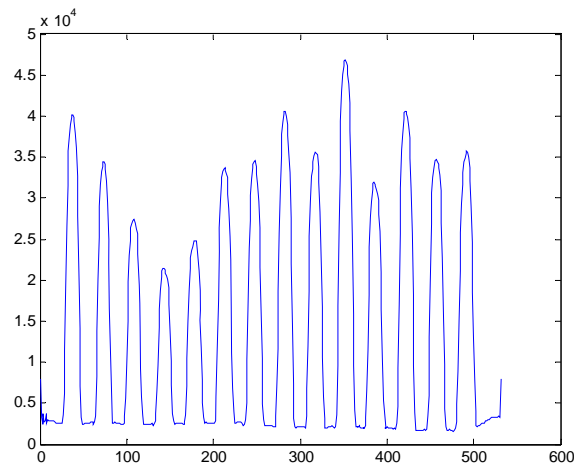


Fig. 6. Vertical projection profile

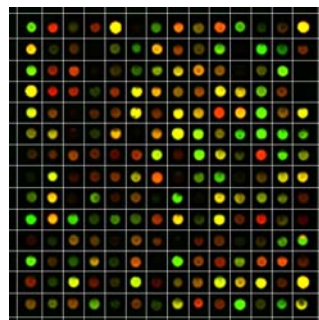


Fig. 7. Result of automatic gridding

The result of automatic gridding algorithm using projection profile for figure 3 are 15 horizontal and 15 vertical line as shown in figure 7. This algorithm is efficient in time computing, in MATLAB this algorithm needs approximately 8 until 9 second for computing automatic gridding for figure 3.

3 Conclusion

First step in microarray image analysis is addressing, that is finding areas in the image on which contain one spot using grid lines. This step is one of the most important step in microarray image analysis and can be done by either manually or automatically. The Gridclus algorithm is an algorithm for microarray image automatic gridding base on k-mean clustering has been presented, but this algorithm is not efficient in time computing. Another algorithm uses mathematical morphology, including image projection profile and other complex mathematical morphology operations. An efficient and simple automatic gridding for microarray image analysis using image projection profile has introduced. This algorithm base on fact that microarray image has local minimum and maximum intensity at background and foreground areas respectively.

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