Granulometric Analysis of Spots in DNA Microarray Images

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As the topological properties of each spot in DNA microarray images may vary from one another, we employed granulometries to understand the shape-size content contributed due to a significant intensity value within a spot. Analysis was performed on the microarray image that consisted of 240 spots by using concepts from mathematical morphology. In order to find out indices for each spot and to further classify them, we adopted morphological multiscale openings, which provided microarrays at multiple scales. Successive opened microarrays were subtracted to identify the protrusions that were smaller than the size of structuring element. Spot-wise details, in terms of probability of these observed protrusions, were computed by placing a regularly spaced grid on microarray such that each spot was centered in each grid. Based on the probability of size distribution functions of these protrusions isolated at each level, we estimated the mean size and texture index for each spot. With these characteristics, we classified the spots in a microarray image into bright and dull categories through pattern spectrum and shape-size complexity measures. These segregated spots can be compared with those of hybridization levels.

Key words: microarray, spot, mathematical morphology, image

Introduction

Recent advances in human genetics have enormous implications for the future (1, 2). The new knowledge has created several scientific fields such as bioinformatics, pharmacogenomics, and others. Bioinformatics, also called biological computing, includes the storage, retrieval, and comparison of DNA sequences within the human genome and between genomes of different species. It is a field that offers various tools and techniques to deal with, in particular, data analysis problems, genomics, proteomics, medical informatics, computational biology, and many others (3). The tools of bioinformatics are powerful computers and sophisticated software used to manage and analyze the biological gene data. One of the recent advances in the field of bioinformatics is the analysis of gene expression levels in microarrays using image processing techniques. DNA microarrays allow researchers to compare the activities of thousands of genes in normal and cancer cells. It is known that cancer disease exhibits altered patterns of gene expression. DNA microarrays have the potential to identify genes that can be targeted by therapeutic drugs. A typical microarray image consists of a few hundreds to several thousands of spots. The extent of hybridization of these spots determines the level of gene expression in the sample. DNA array forms an orderly arrangement of samples for examining gene expression and is known as gene expression array. These gene chips are paradoxically referred to as microarrays.

Microarray chips consist of various spots with varied degrees of luminescence. The motivation for the present investigation is from the fact that the luminescence property of each spot explains the hybridization level. Present investigation aims to classify DNA microarray images based on various structural and morphological characteristics. DNA microarray image analysis is an important topic from the point of view of bioinformatics. Through applications of image analysis techniques, several studies addressing microarray image classifications were carried out earlier (3–10). A microarray consisting of several spots with varied gene expression levels is a gray level image. The gene expression level of a spot can be observed...
through its luminescence. In other words, the brightness, which is expressed in terms of gray level of each spot, needs to be investigated by image processing tools. However, a microarray has spots of several categories ranging from very dull to very bright luminescence. The higher the brightness, the higher the gene expression level, and vice versa. To classify these spots automatically, one of the potential techniques is the pattern spectrum procedure. It is proposed to implement the pattern spectrum procedure on an available DNA microarray image for the purpose of classifying its spots.

In this work, we investigated DNA microarray images based upon the luminescence characteristics using morphological tools. These processed images were analyzed for changes during the processing phases. The changes were quantified using parameters that reflect the shape and size of the spots being analyzed. They exhibited a unique pattern during processing for spots with different luminescence.

**Basic Morphological Transformations**

In order to understand the granulometric analysis, we briefly explain the basic morphological transformations such as erosion, dilation, opening, and closing. Morphological operations will be performed on the grayscale image \( f \) by means of a binary structuring element \( B \). The structuring element \( B \) is like a matrix of size \( n \times n \) \((n \in \text{integers})\).

Erosion of an image \( f \) by octagon \( B = (f \ominus B) \) (1)
Dilation of an image \( f \) by octagon \( B = (f \oplus B) \) (2)
Opening of an image \( f \) by octagon \( B = (f \ominus B) = [(f \ominus B) \oplus B] \) (3)
Closing of an image \( f \) by octagon \( B = (f \bullet B) = [(f \oplus B) \ominus B] \) (4)

Octagon of size \( n = B_n \)
\[ B = B \oplus B \oplus B \ldots \oplus B \ (n \text{ times}) \] (5)

Multiscale transformations of the four basic types given in (1)–(4) are expressed as:

\[
\begin{align*}
\text{Erosion} : & \quad (f \ominus B_n) \\
\text{Dilation} : & \quad (f \oplus B_n) \\
\text{Opening} : & \quad [(f \ominus B_n) \oplus B_n] \\
\text{Closing} : & \quad [(f \oplus B_n) \ominus B_n] \\
\end{align*}
\] (6)

By following a specific criterion, image \( f \) will be transformed by convoluting it with the structuring element \( B \). Morphological erosion is nothing but replacing a center pixel with the minimum intensity value from the neighborhood positions where there will be 20 neighborhood values. We consider an octagonal type of element of size 5x5 (Figure 1C). Performing this operation twice with similar characteristic information of the structuring element gives the effect of morphological erosion of the image by structuring element of size 9x9. The reason behind choosing an octagonal template is that it is a circular disk in eight-connectivity grid \((11)\). This transformation can be iteratively performed to achieve multi-scale morphological transformation. Morphological erosion and dilation will make the image darker and brighter respectively. Increasing the levels of iteration can increase the area extents of these zones. The combination of morphological erosion followed by dilation forms a transformation called opening. This opening transformation can be performed with increasing size of structuring element to have the effect of multi-scale opening. By systematically increasing the size of the structuring element with equal intervals, granulometries can be performed. For more details on the basic morphological transformations and their wide-ranging applications, readers may refer to Serra \((12)\).

To unravel several important spot characteristics, we employed these morphological transformations in a systematic way on a sample microarray image (Figure 1A) with an aim to compute spot-wise pattern spectrum \(\gamma\), in other words, gray level granulometries and shape-size complexity measures. With these characteristics, we classified the spots in a microarray. In the section that follows we provide a simple but elegant framework, based on mathematical morphology, to unravel various morphological characteristics of microarray spots.

**Proposed Method of Analysis**

We recorded the spatial coordinates of each spot embedded within a grid that we placed on the microarray
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Fig. 1 A. DNA microarray image. Spots with different luminescence expressing varied hybridization or gene expression levels are conspicuous; B. Spots with dividing grid lines superposed to understand characteristics of each spot forming a sub-image; C. primitive octagon template of size 5×5, which is considered as $B_n$, $n = 1$.

We understood that the higher the dynamic range, the higher the hybridization level. Each spot in this image (Figure 1B) was appropriately indexed from the spot starting from the left to the right and from the top to the bottom. In other words, an equally spaced square grid was placed on the spots such that each spot was symmetrically embedded within the grid. The coordinates of each grid were considered as the spatial coordinates of a sub-image consisting of a spot. This exercise facilitated to estimate spot-wise gray level distribution and other measures. We performed gray scale granulometries on the entire image by employing an octagonal type of structuring element (Figure 1C). Probabilistic size distribution and its density function were computed to estimate the contributing area due to a significant gray level.

To perform granulometries, the opening transformation is employed. Structuring element ($B$) in this work is a matrix of primitive size 5×5 (Figure 1C). $B$ is of octagon shape and is symmetric with respect to the origin. The emphasis in this section is to develop a framework to understand the gene expression levels that are depicted in the form of varied luminescence values in each sub-image. To avoid extensive computations where it may require the analysis of 256 gray levels since we considered an 8-bit microarray chip, the microarray image of size 810×470 pixels was transformed into multiscale images by performing iterative openings up to 20 cycles by means of a binary octagonal structuring element. The reason for performing opening up to 20 cycles is due to the fact that after 20 cycles of iterative openings, the spots in the microarray image are vanished. To compute the pattern spectrum for each spot and the whole microarray image, we subtracted the succeeding levels of opened microarray images by taking the algebraic difference among these images as below:

$$ (f \circ B_{n-1}) - (f \circ B_n) $$

(7)

The non-zero pixels that are lost from successive levels of opened microarray images provide areas (A). These areas provide the basis to construct pattern spectrum values by means of octagonal element, which we express as

$$ A [(f \circ B_{n-1}) - (f \circ B_n)] $$

(8)

Let $f$ represent a gray level image of DNA microarray consisting of several spots with different luminescence. To compute probabilistic size distribu-
tion and probability size density functions, we performed multi-scale opening by increasing the size of the binary structuring element iteratively up to “$n$” number of times (Figures 2A–U). In these figures, the evolution of spots under the influence of multiscale opening is obvious. Figures 2I–U show significant different information from that of Figures 2A–H, which is due to the fact that certain spots that possess varied gray level compositions are filtered out. This process is also called granulometry that is shown as:

$$f \circ B_n,$$  \hspace{1cm} (9)

where $n$ ranges from 0 to $N$.

From these granulometries, we compute pattern spectrum (PS) as

$$PS_f = A [(f \circ B_{n-1}) - (f \circ B_n)], \hspace{1cm} (10)$$

where $n$ is greater than or equal to 0.

Eq. (10) implies that the PS equals the area occupied by the image obtained by subtracting the image opened using $B_{n+1}$ from the one opened using $B_n$. This subtraction is nothing but the algebraic difference between the two images. We then computed pattern spectrum values for each spot (Figure 1B).
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G. Microarray image after 6 openings
H. Microarray image after 7 openings
I. Microarray image after 8 openings
J. Microarray image after 9 openings
K. Microarray image after 10 openings
L. Microarray image after 11 openings
M. Microarray image after 12 openings
N. Microarray image after 13 openings
Fig. 2 A–U. Granulometric analysis of spots after respective cycle of gray level openings performed by means of octagonal structuring element. The brightness and contrast properties of I–U are readjusted for better legibility.
The four parameters that we computed for each spot include pattern spectrum, probability function, average size, and average roughness. Probability function is an important estimate that we computed by following a simple framework based on multiscale opening, algebraic difference between the two functions, total area of the sub-image consisting of a spot, and pattern spectrum. The algebraic difference between two functions is the difference between the image opened by \( n \)th size of structuring element \((B_n)\) and the one opened by \((n+1)\)th size. The information that is lost will be attained through this algebraic difference. The information loss was quantified through number of pixels (Figures 3A–T). We term this algebraic difference as pattern spectrum. The pattern spectrum at respective opening cycles is employed to compute the probability function as below:

\[
p_{\lambda} = \frac{p_x(k, B)}{A(X)}, \tag{11}
\]

where \( p_{\lambda} = \text{probability function} \),
\( p_x = \text{pattern spectrum} \),
\( A(X) = \text{area of the original function (original image size)} \),
\( B = \text{structuring element} \),
\( K = \text{size of the structuring element} \).

Further, we estimate average size and average roughness for each spot by employing these probability functions as follows:

Average size:

\[
\bar{n} (f/B) = \sum_{n=0}^{N} np_{\lambda} (n), \tag{12}
\]

Average roughness:

\[
H (f/B) = -\sum_{n=0}^{N} p_{\lambda} (n) \log [p_{\lambda} (n)] \tag{13}
\]
Fig. 3 A–T. The images that were obtained by subtracting the respective level of opened spot images from the preceding level. The different images were achieved by simple algebraic difference.
To summarize, this whole study provides a simple framework to classify DNA microarray spots into several categories. The steps of the proposed method are given in Figure 4.

**Fig. 4** Steps of the proposed method of analysis.

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**Results and Discussion**

Spot-wise (Sub-image-wise) pattern spectrum values were computed for all 240 spots in the microarray image by computing the area of non-zero pixels (A) that existed in the respective subtracted images. Equation (8) enables this step. A sequence of these subtracted images is illustrated in Figure 3 (A–T). To have a better visibility, the subtracted images are equalized histograms. Table 1 depicts spot-wise spatial coordinates, average size, and average roughness. By employing equations (11)–(13), we estimated respectively the probability functions, average size, and average roughness for each spot.

On physical correlation, it is observed that the spots with higher luminescence have higher roughness. It may be inferred that the higher the roughness of a spot, the higher the luminescence and thus the hybridization or gene expression level. Spot-wise average size and roughness values are graphically represented for better legibility (Figures 5A and 5B). A simple double-logarithmic graph between average size and average roughness for all the 240 spots is shown in Figure 5C. It is observed that brighter spots form a cluster on positive side (above the horizontal axis) of the graph. By employing these roughness and size values, one can classify these spots using a threshold limit in an automated manner. From Table 1, we can infer that spots with roughness values between 0.8 and 1.1 represent bright ones.

From this analysis, the larger values of average size estimated by the pattern spectrum procedure indicate spots with higher luminescence characters. Meanwhile, roughness values would provide a basis to understand the topology-based classification. The bright spots that are obvious from Figure 1A evidently possess average roughness values in the range of 0.8 and 1.1. Other spots possessing the values beyond this
### Table 1: Spot-wise Average Size and Average Roughness Parameters Computed Through the Pattern Spectrum Procedure

| No. | Spatial Coordinates | Average | Average | | | | Size | Roughness |
|-----|---------------------|---------|---------|---|---|---|---|---|
| 1   | 4 44 7 49           | 4.73    | 0.49    | | | | | |
| 2   | 4 44 89             | 5.11    | 0.51    | | | | | |
| 3   | 4 44 127            | 5.12    | 0.52    | | | | | |
| 4   | 5 43 107            | 4.91    | 0.39    | | | | | |
| 5   | 5 43 187            | 5.13    | 0.52    | | | | | |
| 6   | 7 43 247            | 4.75    | 0.42    | | | | | |
| 7   | 4 44 286            | 4.39    | 0.42    | | | | | |
| 8   | 4 44 326            | 10.00   | 1.04    | | | | | |
| 9   | 3 45 367            | 59.60   | 2.83    | | | | | |
| 10  | 4 43 405            | 12.20   | 1.22    | | | | | |
| 11  | 5 42 443            | 5.00    | 0.51    | | | | | |
| 12  | 4 44 485            | 61.90   | 2.84    | | | | | |
| 13  | 4 44 523            | 11.60   | 1.09    | | | | | |
| 14  | 4 44 563            | 5.24    | 0.51    | | | | | |
| 15  | 5 44 602            | 5.25    | 0.53    | | | | | |
| 16  | 7 44 642            | 7.43    | 0.36    | | | | | |
| 17  | 5 44 683            | 4.18    | 0.45    | | | | | |
| 18  | 3 45 722            | 5.12    | 0.51    | | | | | |
| 19  | 2 43 763            | 5.00    | 0.51    | | | | | |
| 20  | 2 43 801            | 4.79    | 0.46    | | | | | |
| 21  | 4 44 849            | 5.40    | 0.52    | | | | | |
| 22  | 4 44 877            | 5.77    | 0.52    | | | | | |
| 23  | 4 44 127            | 5.72    | 0.56    | | | | | |
| 24  | 4 44 168            | 11.00   | 1.18    | | | | | |
| 25  | 4 44 207            | 69.30   | 2.82    | | | | | |
| 26  | 4 44 248            | 12.70   | 1.20    | | | | | |
| 27  | 4 44 285            | 7.44    | 0.35    | | | | | |
| 28  | 4 44 325            | 59.00   | 2.75    | | | | | |
| 29  | 4 44 365            | 181.00  | 0.94    | | | | | |
| 30  | 4 44 405            | 75.00   | 2.75    | | | | | |
| 31  | 4 44 444            | 60.00   | 2.74    | | | | | |
| 32  | 4 44 485            | 180.00  | 0.92    | | | | | |
| 33  | 4 44 523            | 76.40   | 2.68    | | | | | |
| 34  | 4 44 566            | 4.76    | 0.47    | | | | | |
| 35  | 4 44 602            | 14.30   | 1.15    | | | | | |
| 36  | 4 44 642            | 58.20   | 2.72    | | | | | |
| 37  | 4 44 682            | 4.87    | 0.46    | | | | | |
| 38  | 4 44 722            | 5.86    | 0.54    | | | | | |
| 39  | 4 44 761            | 5.70    | 0.53    | | | | | |
| 40  | 4 44 802            | 8.87    | 0.99    | | | | | |
| 41  | 4 44 849            | 5.41    | 0.51    | | | | | |
| 42  | 4 44 882            | 5.02    | 0.49    | | | | | |
| 43  | 4 44 127            | 61.90   | 2.79    | | | | | |
| 44  | 4 44 167            | 181.00  | 0.94    | | | | | |
| 45  | 4 44 207            | 81.10   | 2.64    | | | | | |
| 46  | 4 44 246            | 81.10   | 2.64    | | | | | |
| No. | Spatial Coordinates | Average | Average Size | Roughness |
|-----|---------------------|---------|--------------|-----------|
|     | Xmin | Xmax | Ymin | Ymax |         |        |
| 93  | 157  | 193  | 483  | 523  | 103.00  | 2.53   |
| 94  | 159  | 194  | 523  | 565  | 24.80   | 1.81   |
| 95  | 157  | 194  | 564  | 602  | 5.72    | 0.53   |
| 96  | 155  | 193  | 603  | 643  | 9.19    | 1.04   |
| 97  | 156  | 194  | 642  | 683  | 41.40   | 2.52   |
| 98  | 156  | 194  | 682  | 721  | 178.00  | 1.06   |
| 99  | 156  | 195  | 721  | 762  | 99.70   | 2.51   |
| 100 | 156  | 195  | 761  | 803  | 6.36    | 0.68   |
| 101 | 195  | 232  | 8    | 47   | 13.60   | 1.28   |
| 102 | 197  | 232  | 48   | 83   | 74.90   | 2.38   |
| 103 | 195  | 232  | 88   | 127  | 21.60   | 1.59   |
| 104 | 196  | 232  | 127  | 168  | 5.66    | 0.55   |
| 105 | 195  | 232  | 166  | 208  | 5.72    | 0.53   |
| 106 | 192  | 231  | 206  | 246  | 5.23    | 0.48   |
| 107 | 194  | 233  | 246  | 285  | 4.95    | 0.49   |
| 108 | 194  | 233  | 288  | 325  | 5.32    | 0.50   |
| 109 | 194  | 231  | 326  | 364  | 59.30   | 2.74   |
| 110 | 195  | 233  | 367  | 407  | 64.70   | 1.07   |
| 111 | 194  | 231  | 404  | 446  | 141.00  | 2.08   |
| 112 | 195  | 231  | 444  | 488  | 181.00  | 0.95   |
| 113 | 195  | 232  | 484  | 524  | 77.60   | 2.70   |
| 114 | 195  | 231  | 524  | 566  | 4.67    | 0.45   |
| 115 | 193  | 230  | 564  | 603  | 5.11    | 0.47   |
| 116 | 192  | 232  | 603  | 645  | 45.50   | 2.52   |
| 117 | 194  | 231  | 644  | 685  | 11.00   | 1.13   |
| 118 | 193  | 231  | 682  | 721  | 78.80   | 2.55   |
| 119 | 193  | 231  | 720  | 760  | 37.50   | 2.16   |
| 120 | 194  | 232  | 763  | 801  | 5.54    | 0.56   |
| 121 | 232  | 269  | 8    | 49   | 5.49    | 0.55   |
| 122 | 229  | 268  | 49   | 87   | 10.10   | 1.07   |
| 123 | 233  | 269  | 110  | 89   | 125.00  | 1.85   |
| 124 | 232  | 269  | 130  | 165  | 23.00   | 1.61   |
| 125 | 232  | 271  | 168  | 208  | 69.00   | 2.69   |
| 126 | 232  | 268  | 205  | 247  | 14.50   | 1.28   |
| 127 | 232  | 268  | 249  | 287  | 5.74    | 0.55   |
| 128 | 232  | 266  | 287  | 322  | 5.35    | 0.44   |
| 129 | 231  | 269  | 324  | 367  | 17.70   | 1.48   |
| 130 | 233  | 268  | 403  | 430  | 30.50   | 2.04   |
| 131 | 231  | 269  | 408  | 444  | 36.60   | 1.94   |
| 132 | 232  | 269  | 444  | 481  | 102.00  | 2.36   |
| 133 | 230  | 268  | 483  | 524  | 30.70   | 1.97   |
| 134 | 231  | 272  | 524  | 563  | 68.60   | 2.65   |
| 135 | 233  | 276  | 563  | 605  | 12.60   | 1.21   |
| 136 | 233  | 268  | 602  | 643  | 12.50   | 1.24   |
| 137 | 232  | 270  | 641  | 685  | 4.48    | 0.50   |
| 138 | 231  | 270  | 683  | 724  | 9.27    | 1.02   |
| 139 | 233  | 267  | 720  | 759  | 58.10   | 2.65   |
Table 1  Continued

| No. | Spatial Coordinates | Average | Average Roughness |
|-----|---------------------|---------|-------------------|
|     | Xmin Xmax Ymin Ymax | Size    | Roughness         |
| 187 | 344 382 246 285     | 116.00  | 1.82              |
| 188 | 342 380 289 326     | 21.70   | 1.65              |
| 189 | 346 385 326 366     | 69.80   | 2.69              |
| 190 | 342 381 367 404     | 12.90   | 1.18              |
| 191 | 342 379 404 443     | 17.10   | 1.48              |
| 192 | 343 383 443 483     | 87.70   | 2.59              |
| 193 | 345 379 484 520     | 24.40   | 1.73              |
| 194 | 343 382 525 564     | 5.47    | 0.54              |
| 195 | 346 380 563 602     | 64.70   | 2.77              |
| 196 | 340 381 602 643     | 144.00  | 1.43              |
| 197 | 345 382 642 683     | 4.58    | 0.47              |
| 198 | 344 381 682 721     | 8.15    | 0.89              |
| 199 | 346 381 722 763     | 66.50   | 2.63              |
| 200 | 343 382 761 802     | 24.20   | 1.75              |
| 201 | 382 418 9 46        | 5.24    | 0.46              |
| 202 | 382 417 15 89       | 38.20   | 2.47              |
| 203 | 382 419 88 129      | 160.00  | 0.94              |
| 204 | 382 419 128 166     | 79.50   | 2.69              |
| 205 | 382 419 169 204     | 5.92    | 0.52              |
| 206 | 381 418 206 246     | 52.90   | 2.67              |
| 207 | 384 418 247 286     | 163.00  | 0.86              |
| 208 | 382 418 288 327     | 104.00  | 2.05              |
| 209 | 381 420 325 364     | 155.00  | 1.16              |
| 210 | 381 419 365 404     | 78.50   | 2.57              |
| 211 | 381 419 406 445     | 4.97    | 0.46              |
| 212 | 384 419 445 485     | 14.70   | 1.28              |
| 213 | 383 419 486 524     | 72.80   | 2.58              |

Fig. 5  A. Graph showing spot-wise average sizes.  B. Graph showing spot-wise average roughness values.  C. Log-log graph between average size and average roughness.
range possess significantly different roughness values. The larger the average size, the larger the size of octagonal element in a spot. From this fact, we infer that these spots consist of larger size of octagonal element. A graph is plotted with spot numbers on X-axis and average roughness on Y-axis (Figure 5B). Similarly, higher average size values of more than 145 were observed for the bright spots with 29, 32, 39, 45, 49, 56, 69, 73, 82, 94, 99, 110, 111, 112, 117, 172, 179, 196, 203, and 233. The corresponding roughness values were observed in the range of 0.8 and 1.1. It is inferred from this unsupervised classification that the spots with higher average size values, which are also bright spots, possess the roughness values between 0.8 and 1.1. This study, which was carried out in unsupervised way, needs further supporting evidence that is possible if the results derived from core microbiological analysis are available.

Conclusion

This study provides a simple framework to classify DNA microarray spots based upon their gene expression levels. This work demonstrates possibility to unravel topological characteristics of microarrays using powerful computers and established tools like mathematical morphology. These characteristics provide new insights in understanding microbiological phenomena in a quantitative manner.

A brief idea about the gist of this work is to identify the presence of gene expression level in the desired DNA sequence. The DNA sequence is converted into a microarray image, which is a gray level image depicting gene expression levels in terms of intensities. Analysis of this microarray image is performed by using concepts from mathematical morphology and computational tools. Then, the spots in the image are categorized based on the average roughness values into bright and dull categories. The motivation for this work is to show a relation between the roughness value and the brightness of a spot. From this study, we can infer that the average roughness is proportional to the brightness or luminescent property of a spot, which is proportional to its gene expression level or hybridisation level. From the sample microarray spot image considered, based on this approach, the salient features observed include:

1. Larger values of average size estimated by the pattern spectrum procedure indicate spots with higher luminescence characters.

2. Roughness values would provide a basis to understand the topology-based classification.

3. Bright spots that are obvious from Figure 1A evidently possess average roughness values in the range of 0.8 and 1.1, while spots beyond this range possess significantly different roughness values.

4. The higher the average size, the higher the size of octagonal element that a spot consists of.

5. Higher average size values of more than 145 are observed for the bright spots with 29, 32, 39, 45, 49, 56, 69, 73, 82, 94, 99, 110, 111, 112, 117, 172, 179, 196, 203, and 233.

Using morphology, the rich topological properties extracted from DNA chips are immensely valuable to cell biologists and scientists who study the roots of cancer and other complex diseases. The application areas include the research on gene function, gene pathways, disease classifications, and disease origin. The parameters (shape-size complexity measures) that we computed may change as we change the characteristic information of the structuring element. By changing the structuring element or template, we plan to characterise the spots in our future studies to generate a spectrum of these topological parameters.

Materials and Methods

Array fabrication

Array fabrication involved preparing a glass slide, obtaining the DNA sequence representing genes of a genome of interest, and depositing (printing) the cDNA sequences on the glass slide. A cDNA sequence, called a cDNA probe, was selected to make the arrays. In order to get this cDNA sequence, one requires cDNA clones and cDNA library. Each cDNA clone was amplified to get many copies using the PCR (polymerase chain reaction) technique. After amplification, the PCR product (the liquid containing the amplified cDNA probes) was deposited on the polysylne-coated glass slide using a set of microspoting pins. A typical example was the spotting of more than 6,000 different PCR generated DNA samples on a polysylne-coated slide measuring 18×18 mm². The drops of solution containing cDNA probes formed the spots on the array.

Sample preparation

The experimental material was a tissue sample from a patient. From the cell, total RNA was extracted and
out of which mRNA was isolated. This mRNA was converted into a more stable cDNA by the process of reverse transcription. Thus, one pool of cDNA was prepared from the experimental sample. In the same way, another pool of cDNA was prepared from the normal cell. Hence, for the preparation of microarrays, two pools of cDNA were synthesized.

**Experimental and reference target cDNAs’ labeling**

The cDNAs obtained from experimental mRNA were labeled with red fluorescent dye Cy5 and those from reference sample were labeled with green fluorescent dye Cy3. These are called target cDNAs.

**Hybridization**

Hybridization refers to the binding of two complementary DNA strands by base pairing. The mixed solution of experimental and reference target cDNAs was applied to the array, which contained the probe cDNA in each spot. A specific spot on the array contained cDNA probes for gene A. The target cDNA in the mixed solution, which was complementary to the probe cDNA of gene A, bound together by base-pairing which was nothing but hybridization.

**Microarray quantification**

The expression levels of a gene in the experimental and reference cells were measured by the spot intensities of the Cy5 (red) dye and Cy3 (green) dye respectively. Dyes or fluorescent intensities were obtained by scanning the array using a confocal laser microscope. The products resulting from the array scanning process were two 16-bit tagged image file format (TIFF) images. The scanned array area was divided into equally sized pixels and the resulting image contained fluorescence intensities for corresponding pixels.

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