Original Article

# Microarray Images Contrast Enhancement and Gridding Using Genetic Algorithm

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## Abstract

**Background:** Microarray is a sophisticated tool that concurrently analyzes the expression levels of thousands of genes, giving scientists an overview of DNA and RNA study. This procedure is divided into three stages: contact with biological samples, data extraction, and data analysis. Because expression levels are disclosed by the interplay of light with fluorescent markers, the data extraction stage relies on image processing methods. To extract quantitative information from the microarray image (MAI), four steps of preprocessing, gridding, segmentation, and intensity quantification are required. During the generation of MAIs, a large number of error-prone processes occur, leading to structural problems and reduced quality in the resulting data, affecting the identification of expressed genes. **Methods:** In this article, the first stage has been examined. In the preprocessing stage, the contrast of the images is first enhanced using the genetic algorithm, then the source noises that appear as small artifacts are removed using morphology, and finally, to confirm the effect of the contrast enhancement (CE) on the main stages of microarray data processing, gridding is checked on complementary deoxyribonucleic acid MAIs. **Results:** The comparison of the obtained results with an adaptive histogram equalization (AHE) and multi-decomposition histogram equalization (M-DHE) methods shows the superiority and efficiency of the proposed method. For example, the image contrast of the Genomic Medicine Research Center Laboratory dataset is 3.24, which is 42.91 with the proposed method and 13.48 and 32.40 with the AHE and M-DHE methods, respectively. **Conclusions:** The performance of the proposed methods for CE is evaluated on 3 databases and a general conclusion is obtained as to which CE method is more suitable for each dataset.

**Keywords:** *Contrast enhancement, genetic algorithm, genomics, gridding, mathematical morphology, microarray images*

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## Introduction

In the early 1990s, microarray technology was able to create a great revolution in genomics and made it possible to examine the gene profile of thousands of genes simultaneously. This technology represents a scientific intersection between life science and an electronic device that analyzes gene information on a large scale[1] and plays an important role in biological conclusions. The primary purpose of microarray studies is to identify gene expression patterns to derive relevant biological inferences and solve basic experimental questions.[2] They enable users to compare gene expression in various circumstances, cells, and even tissues.[3] This potential expands our understanding of the fundamental components of life's growth and development, as well as the genetic reasons for anomalies in the human body.

The raw image of the complementary deoxyribonucleic acid (cDNA) microarray taken from the scanner needs preprocessing, during which the values of the common fluorescent intensity of each spot can be accurately determined. The scanner's resolution is an important parameter in determining the quality of the microarray image (MAI) so if the contrast of the MAI is low, the quality of the edges extracted from the image will be poor.[4] The information on these edges is the primary source for gridding of MAIs. Due to the low contrast of the MAI, it is challenging to tell the foreground spots from the equivalent background, and hence, contrast enhancement (CE) is crucial in image processing. Therefore, CE is required to draw attention to key elements in MAI



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data.[5] An image with good contrast will have distinct sections that can be seen.

The contrast and quality of these images have recently been improved using a variety of techniques. Adaptive histogram equalization (AHE)[6] is the most straightforward and practical of all. In addition, in some local enhancement techniques, such as the use of multiscale morphological procedures to generate image representation, distinct CE operators are employed in the spatial domain (multiscale) or frequency (multiresolution).[7] Other sources include fuzzy clustering[8] and multi-decomposition histogram equalization (M-DHE).[9] Wavelet transform has been adopted to realize CE. These methods usually involve the parametersetting and threshold search. Improving MAI quality aims to improve accuracy of the processing steps such as gridding or segmentation[10]. Due to the aforementioned issues, we suggested a preprocessing step for the MAI. In this study, a brand new, entirely automatic technique for identifying and enhancing image contrast is put forth. Unlike earlier techniques, it does so without spatial or frequency domain image segmentation, increasing image contrast. The genetic algorithm (GA), which solely considers entropy when determining the fitness function, is employed[11] to increase contrast; however, we are aware that the MAI has the property that its spots have greater gray values while the background has lower gray values.[12] In this article, a method based on a GA for CE is introduced, which successfully handles various image kinds without altering any parameters. The approach generates the fitness function using 10 indices defined on the contrast of the image.

In addition, the contamination of MAIs with different types of noise brought on by biological and experimental factors is a significant component that makes feature extraction and analysis more difficult. The presence of noise results in inaccurate spot segmentation, which can therefore compromise the repeatability and validity of the gene expression level determined by the MAIs and result in inaccurate calculation of the average relative intensity of the spots. The wrong conclusion may be caused by ignoring or mishandling the MAIs with noise. Experimental noise is divided into source noise and detector noise. Source noise is generated during target construction and labeling, while detector noise is generated during amplification and digitization steps. Examples of source noise include discrete image artifacts in the image such as large fluorescent dust particles, independent staining, salt deposition from evaporated solvents, filaments, and various air debris.[13] These types of noises do not have a specific pixel value, and according to the way they appear and their nature, they affect the contrast of the image and reduce the quality of the edges of the spots. In recent years, methods such as wavelet transform, mathematical morphology, and spatial filters such as median and Wiener led to noise reduction and shape improvement in medical and MAIs.[14] Hence, spatial filters use spatial masks to process digital images and are divided into linear and nonlinear. The median filter is the simplest nonlinear filter whose main transfer function calculates the average pixel brightness value in the neighborhood where the filter is placed and is quite effective in reducing impact noise.[15] However, because the artifacts have different spatial shapes with different brightness, mathematical morphological methods have better results to remove them. Therefore, in this article, to enhance the contrast, a morphological method is used to remove the artifacts, and in the final stage, these algorithms are combined with the GA method to enhance the final contrast. Metaheuristic algorithms have not been used to enhance the contrast of MAIs, and a GA is used for this purpose for the first time in this article. Furthermore, considering the effect of artifacts on image contrast and combining GA with morphology is proposed for the first time.

This article is as follows: Section 2 describes the proposed methods and Section 3 presents experimental results, discussion, and comparison. The conclusion is summarized in section 4.

## Materials and Methods

The ultimate goal of MAI analysis is to provide useful results that aid in medical diagnosis. To achieve this goal, this paper will focus on appropriate MAI processing techniques that perform preprocessing and gridding to cover effective spot segmentation and ultimately significantly increase the accuracy of correct spot detection to achieve a good estimation of gene expression level.[16] Considering that different images have different degrees of contrast, at first, a fourth-order moment is used to calculate the contrast of the image. The second step achieves CE with a GA for low-contrast images. In the next step, the small noises of the source as artifacts are reduced using mathematical morphology. In the end, to confirm the importance of CE in preprocessing, gridding is performed using the maximum interclass variance method. The steps of the proposed algorithm are shown in Figure 1.

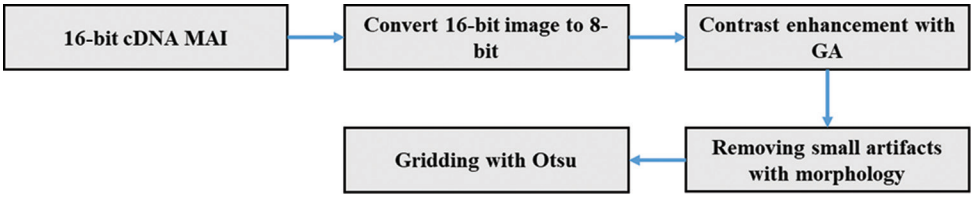


**Figure 1: Block diagram of the proposed method**

## Datasets

The proposed algorithms are implemented on 3 datasets and the results of these datasets are compared with each other. Microarray blocks were stored in the tagged image file format and each pixel in the array has a 16-bit intensity value. Specifications of the MAIs are as follows.

The first dataset contains 56 MAIs from the public Stanford Microarray Database (SMD, http://smd.stanford.edu) that corresponds to Channels 1 and 2 of a microarray experiment; these pairs are the results of four experiments in a study on yeast.

The second dataset is selected from Joe DeRisi's individual dataset (DeRisi) (http://www.bio.davidson.edu/projects/magic/magic). Each image has four blocks, and each block contains 1600 spots. Images corresponding to channels Cy3 and Cy5 have been labeled with the experiment IDs 1302, 1303, 1309, 1310, 1311, 1312, and 1313. The images in this database are related to yeast.

The third dataset is the Changgang Hospital Genomic Medicine Research Center Laboratory database (GMRCL). This database was prepared by the Genomic Medical Research Center of Changgang Hospital and contains 16 MAIs related to cervical cancer. Each array has 32 blocks and 15,488 spots with 7744 genes. The approximate dimensions of all images are 2036 × 3848.

## Contrast Enhancement Using Genetic Algorithm

If the MAI contrast is low, the quality of the edges extracted from the image will be poor. The information on these edges is the primary source for gridding MAIs.[17] The quality of the edges of spots is enhanced using the CE algorithm based on the GA.

At the beginning of the work, image contrast is calculated using a four-order moment according to Eq. (1).[18]

$$c = \frac{SD}{\left[\frac{Fom}{MSE^2}\right]^{1/4}}. \quad Fom = \frac{1}{N}\sum(p - \bar{p})^4 \qquad (1)$$

Where SD is the standard deviation, MSE is the mean square error, Fom is the fourth-order moment, N is the number of image pixels, and $\bar{P}$ is the average value of the image. The main contrast value of images taken from 3 different datasets can be calculated according to Eq. (1). Figure 2 shows the contrast values of the images for 3 datasets.

It can be seen that the image contrast values are very different in different datasets. Even in the same datasets, they are different from each other. The images of the DeRisi dataset have higher values, indicating that they have good contrast. Conversely, the images from the SMD dataset are all at the lowest values, indicating that they have low contrast. The average values of contrast for all datasets are given in Table 1, and the comparison of these values confirms the above results.

MAIs have a very different pixel distribution and use only a smaller range of all possible intensity levels. Histograms of real MAIs have a unimodal distribution, which indicates their poor contrast. The existence of irregular round areas of spots with variable brightness causes sudden changes that create impact noise in the images. Therefore, a GA is used to deal with such characteristics for better CE. The steps of this metaheuristic algorithm are shown in Figure 3.

As can be seen in Figure 3, first of all, the number of chromosomes C and the number of generations N must be initialized. This algorithm will be applied to C chromosomes for N number of times. The accuracy of the algorithm depends on the number of generations. After the parameters are initialized, the GA is applied.

As shown in Figure 3, the number of chromosomes C and the number of generations N must be initialized initially. This procedure will be repeated N times on C chromosomes. The algorithm's accuracy is determined by the number of generations. The GA is applied after the parameters have been set up.

The GA begins with a population that is produced at random. This population is chosen to produce a new generation, which is known as the child chromosome. Mutation and combination are the genetic operators employed. Child chromosomes are created from parent chromosomes by modifying one of the values on each chromosome with a particular step size. The child's chromosomes formed in this manner will have the same number and properties as the parent's chromosomes. A combination operator

**Table 1: Average contrast values for all datasets**

| Datasets | SMD | DERISI | GMRCL |
|---|---|---|---|
| Average contrast | 0.99 | 8.15 | 2.08 |

SMD – Stanford Microarray Database; GMRCL – Genomic medicine research center laboratory database; DERISI – Joe DeRisi's Individual Dataset

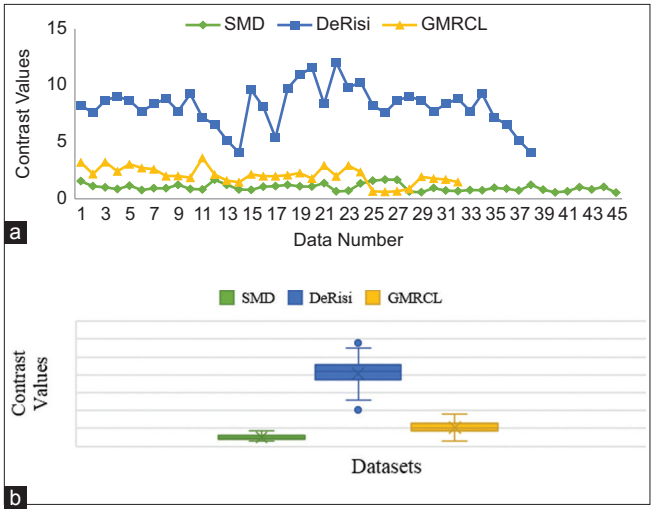


**Figure 2: The contrast values of all data for 3 datasets. (a) Bar chart. (b) Box plot**

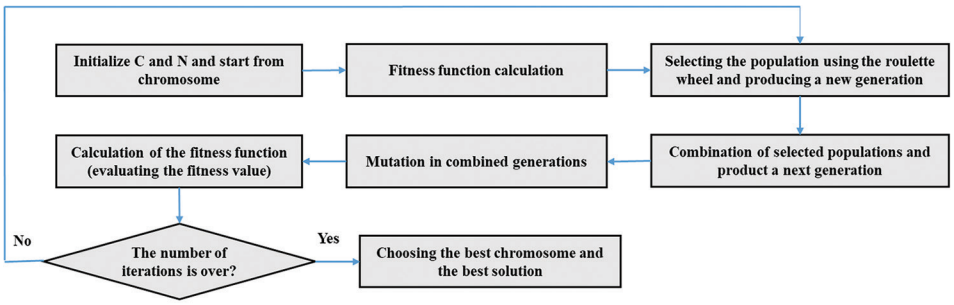


**Figure 3: Block diagram of the genetic algorithm in the proposed method**

generates a child chromosome from more than one parent chromosome. The new chromosome will inherit some characteristics from the first parent and others from the second. If a child's chromosome inherits the finest qualities from both parents, it may outperform both parents. In this situation, each chromosome value intersects with the value of another chromosome.

The fitness value is then calculated in the next step. Each chromosome's fitness value is determined using the fitness function. The chromosome with the highest fitness value is considered the best chromosome and can be utilized more frequently. The fitness function is defined using the weighted summation approach, which produces the fit value by taking into account all of the evaluation criteria. The weighted sum technique gives equal weight to each fitness function parameter.[19] The fitness function is computed as follows:

$$\begin{aligned}\text{Fitness} = &(w_1 \times f_1)+(w_2 \times f_2)+(w_3 \times f_3)+(w_4 \times f_4)\\ &+(w_5 \times f_5)+(w_6 \times f_6)+(w_7 \times f_7)+(w_8 \times f_8)\\ &+(w_9 \times f_9)+(w_{10} \times f_{10})\end{aligned} \quad (2)$$

Hence, w_1, w_2, w_3, …,w_10 are the feature weights, while f_1, f_2, f_3,…, f_10 are the fitness function features and are identical to the following values:

f_1=EMEE, f_2=ME, f_3=MI, f_4=MLI, f_5=CII, f_6=CD, f_7=SD, f_8=MCI, f_9=MSD, f_10=PEOIR

These features are defined as follows:

1. Measure of Enhancement of Entropy (EMEE): it is calculated by calculating the average ratio of maximum to minimum pixel intensity of the enhanced image to the original image

$$\text{EMEE} = \frac{1}{mn}\ln\left(\frac{I_{max}}{I_{min}}\right)\left(\frac{I_{emax}}{I_{emin}}\right) \quad (3)$$

2. Measure of Entropy (ME): it is a measure of randomness

$$\text{ME} = \sum_{i=1}^{m}\sum_{j=1}^{n} p(i.j)\log\_2(p(i.j)) \quad (4)$$

3. The mean intensity (MI) of the image pixels

$$\text{MI} = \frac{1}{mn}\sum_{i=1}^{m}\sum_{j=1}^{n} I(i.j) \quad (5)$$

4. Measure of luminance index (MLI): this is the average ratio of the enhanced image to the original image

$$\text{MLI} = \text{MI}(I_e)/\text{MI}(I_o) \quad (6)$$

5. Measure of Contrast Improvement Index (CII): this index is the enhanced contrast to the original contrast ratio

$$\text{CII} = \frac{C_{enhanced}}{C_{original}}.\ C = (r-b)/(r+b) \quad (7)$$

*R* is the average gray level value of the foreground and b is the average gray level value for the background.

6. Contrast difference (CD): Imax and $I_{min}$ are the maximum and minimum pixel intensities

$$\text{CD} = I_{max} - I_{min} \quad (8)$$

7. Standard deviation (SD)
8. Measure of contrast index (MCI): this ratio is the increased SD compared to the original image. The SD is defined as follows:

$$\text{MCI} = \frac{\sigma I_e}{\sigma I_o} \quad (9)$$

Where, $I = \sqrt{\frac{1}{mn-1}\sum_{i=1}^{m}\sum_{j=1}^{n}\left(I(i.j)-\text{MI}(i,j)\right)}$

9. Mean square difference (MSD): $I_e$ and $I_o$ are the intensity of the enhanced and original images, respectively

$$\text{MSD} = \frac{1}{mn}\sum_{i=1}^{m}\sum_{j=1}^{n}(I_e - I_o)^2 \quad (10)$$

10. Peak enhancement ratio to the original image (PEOIR):

$$\text{PEOIR} = 10\log_{10}\left(\frac{I_{max}^{\ 2}}{\text{MSD}}\right) \quad (11)$$

The best fitness value is chosen during the selection process, and this results in the best chromosome. Chromosomes are only retained if they pass the fitness test. The chromosomes with the highest fitness value are then substituted for those with the lowest fitness value. This step will continue for the N number of C chromosomes.

To achieve the optimal fit, iterations are crucial. The best fit, or high-contrast original picture, expresses the ratio of the input light intensity of the image pixels to the output light intensity of the image pixels. As a result, the algorithm's halting criterion is based on the maximum number of iterations. The program ends after obtaining the chromosome with the highest fitness score. As a result, only one chromosome, which has all of the components for the cDNA MAI sites, is left at the end. In the development of the enhanced image, the data from the chromosome are employed as the best weights.

### Reduction of artifacts using morphology

Mathematical morphology is focused on finding and tracking small noises in the image, and they operate based on the structural characteristics of objects with low computing time. In cDNA MAIs, small artifact pixels are considered holes due to their small area. Hence, morphological filters have a good result in removing these noises. In this article, different morphological operators are combined to achieve desirable performance in contrast to MAIs. For this purpose, the following method is suggested:

In this method, at first, the bright areas resulting from the white top hat transform (WTHT) are added to the original image, and the dark areas resulting from the black top hat transform (BTHT) are subtracted from it. With this method, in addition to eliminating small noises in the foreground, the contrast between the spots and the background is also increased.[20] If the input image is defined as MI ($x_h$, $y_v$), these steps are applied to the images as follows:

$$D(x_h.y_v) = \left(MI(x_h.y_v) + WTHT(x_h.y_v)\right) \text{-}BTHT(x_h.y_v) \quad (12)$$

Hence, $BTHT(X_h.Y_v)$ and $WTHT(X_h.Y_v)$ are the images obtained by applying bottom-hat and top-hat transformations, respectively. This method will be called the Denoising method in the rest of the article.

In this article, the proposed morphological method will be applied separately to all datasets and then combined with the GA and considered as one of the proposed methods of the article. In the results section, the combination of the GA with this method is referred to as GA + Denoising.

### Gridding

The process of determining the location of spots is defined as gridding. Most microarray gridding methods use semiautomatic geometric techniques or complex methods that are computationally expensive. Since typical MAIs contain hundreds or thousands of spots, a practical gridding method should be fully automated, fast, and simple.[21] In this article, gridding is proposed based on the maximum interclass variance,[22] which is carried out in the following steps:

1. Calculation of vertical or horizontal projection signals
2. Filtering the projection signal using morphological reconstruction
3. Finding the optimal threshold using the largest interclass variance
4. Obtaining vertical or horizontal grid lines according to thresholding
5. Calculating the coordinates of the final vectors H and V for all grid lines
6. Determining the number of horizontal grid lines (h) and the number of vertical lines (v).

## Experimental Results

This section aims to present the results of the proposed method and demonstrate their effectiveness in improving MAI contrast. In this section, the results will be presented in two sections. In the first part, the results related to the proposed method for CE are discussed, and in the second part, the effect of the proposed CE method on gridding is stated. The proposed algorithms are implemented on 3 datasets and the results of these datasets are compared with each other.

Table 2 shows the contrast values calculated based on the application of the proposed methods for the original images in 3 datasets and also for the contrast-enhanced images with the proposed methods. A sample image is randomly selected from each dataset as the original image representing that dataset, and then, the contrast values are calculated. For a better comparison, the obtained contrast values are shown as a graph in Figure 4.

According to Table 2 and Figure 4, it can be concluded that the GA alone increases the contrast of the images to a great extent, but removing small artifacts helps to enhance the contrast more efficiently. Because these artifacts have uncertain intensity values when they disappear, they lose their effect on calculating the contrast and improving it, and as a result, they do not cause errors in the CE algorithm. Therefore, it can be seen that their combination with the GA has been very efficient. For example, in the DeRisi dataset, the contrast of the original image is 8.23, and the GA increases this value to 29.39, which is a significant improvement. By applying the GA + Denoising method, the contrast value becomes 33.80. As can be seen, the GA + Denoising method causes more improvement in the

**Table 2: The contrast of the dataset's images with the proposed contrast enhancement methods**

| Datasets | Methods | | | Proposed methods | |
|---|---|---|---|---|---|
| | Original image | AHE[6] | M-DHE[9] | GA | GA + Denoising |
| SMD | 0.82 | 2.95 | 10.92 | 12.46 | 12.75 |
| DERISI | 8.23 | 19.60 | 28.46 | 29.39 | 33.80 |
| GMRCL | 3.24 | 13.48 | 32.40 | 31.46 | 42.91 |

SMD – Stanford Microarray Database; GMRCL – Genomic medicine research center laboratory database; AHE – Adaptive histogram equalization; M-DHE – Multi-decomposition histogram equalization; GA – Genetic algorithm; DERISI – Joe DeRisi's individual dataset

contrast of the images compared to the GA method alone, and this increase is especially significant in SMD and GMRCL datasets.

To investigate the performance of the proposed algorithms, all datasets are also enhanced with the common AHE algorithm[6] and the M-DHE.[9] The contrast values of all datasets enhanced by the AHE method are lower than the values related to the proposed methods. However, M-DHE results in SMD and DeRisi datasets are close to GA results. In the GMRCL dataset, it has a higher than GA but is still lower than the value obtained by the proposed method. This result shows that the proposed methods are more efficient in improving all types of MAIs. For example, in the SMD dataset, the contrast of the original image is 0.82, which increases to 12.46 and 12.75, with the application of GA and GA + Denoising methods, respectively. This is while, the AHE and M-DHE only increase the contrast to 2.95 and 10.92, respectively, which confirms the advantage of the proposed methods to the common ones in the CE.

To select the most efficient CE methods more accurately, in addition to the contrast values, the evaluation indices introduced for the fitness function of the GA in section 2 are considered. These indices include ME, MLI, MI, contrast improvement ratio (CIR), CII, and contrast per pixel (CPP). Table 3 shows the values of these indices when applying 2 proposed methods for 3 datasets. The highest values of the indices for each of the datasets are bolded in the table.

Out of the 17 bolded values in Table 3, 8 indices are related to the application of the GA method and 9 indices are for the GA + Denoising method. Furthermore, the GA method alone performs the best in the SMD and DeRisi datasets, while the GA + Denoising method shows its best performance in the GMRCL dataset.

The CPP values in Table 3 show that in the SMD and DeRisi datasets, the value of GA is the highest. This index is usually used to measure image quality by calculating the contrast value of each pixel. Hence, although the contrast value of GA for this dataset is lower than GA + Denoising, it can maintain the image quality better. MI and MLI indices are also the highest for these 2 datasets with the GA method. Since MI is the average intensity of all pixels in the image and MLI is the ratio of the CE image to the original image, it is used as a luminance index. Hence, when the luminance of the image is important in improving the contrast, we can use GA for this dataset.

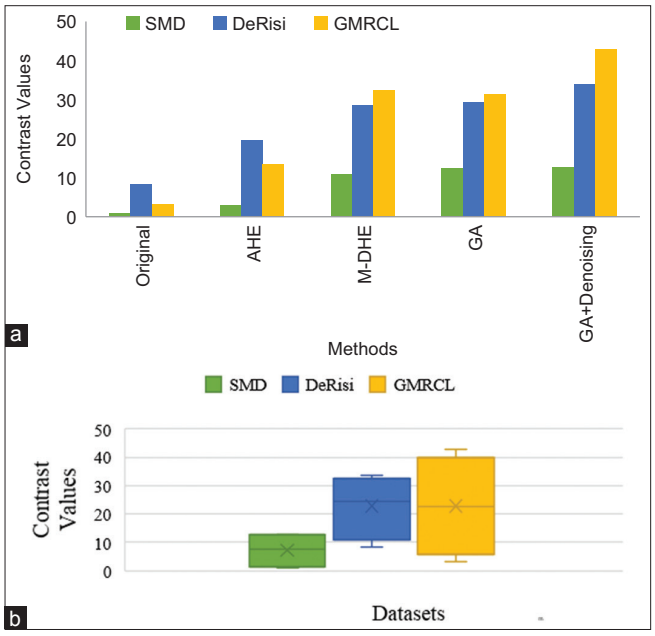


**Figure 4: Comparing the contrast of the dataset's images with the proposed CE methods, (a) Bar chart, (b) Boxplot**

**Table 3: Values of contrast detection indices for the datasets**

| Indices | Datasets | | | | | |
|---|---|---|---|---|---|---|
| | SMD | | DERISI | | GMRCL | |
| | Methods | | | | | |
| | GA | GA + Denoising | GA | GA + Denoising | GA | GA + Denoising |
| CPP | 11.27 | 5.94 | 5.16 | 2.96 | 3.53 | 3.63 |
| CII | 15.18 | 15.53 | 3.57 | 4.11 | 9.71 | 13.24 |
| CIR | - | - | 1.46 | 3.12 | 1.36 | 1.31 |
| MI | 88.43 | 45.59 | 40.79 | 23.18 | 28.23 | 29.04 |
| MLI | 53.76 | 27.72 | 2.90 | 1.65 | 11.63 | 11.97 |
| ME | 1.42 | 1.81 | 4.37 | 2.59 | 1.86 | 2.09 |

SMD – Stanford Microarray Database; GMRCL – Genomic medicine research center laboratory database; GA – Genetic algorithm; MLI – Measure of luminance index; ME – Measure of entropy; CPP - Contrast per pixel; CII - Contrast improvement index; CIR - Contrast improvement ratio; MI - Mean intensity; DERISI - Joe DeRisi's individual dataset

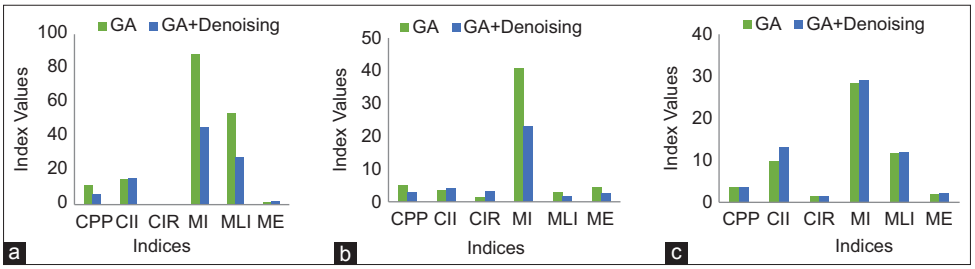


**Figure 5: Comparison of contrast evaluation indices of the (a) Stanford Microarray Database (b) DeRisi (c) Changgang Hospital Genomic Medicine Research Center Laboratory images with the proposed methods**

The ME index, which is an index of entropy and its high value indicates the higher resolution of the image, has the highest value in the DeRisi dataset with the GA method and in the SMD dataset with the GA + Denoising method. This result can also be seen in Figure 5.

Finally, the CII and CIR indices, which are the CE index and the CE ratio, can help us decide on the best method.[23] For this purpose, the CII index values for 3 datasets with 2 proposed methods have been compared with each other in Figure 6. According to this figure and the values in Table 2, it can be concluded that the GA + Denoising method works better than other methods in CE for all datasets. Based on the values of the indices in Table 3, the best value of the fitness function obtained is given in Table 4. These values express the ratio of the input light intensity of the image pixels to the output light intensity of the image pixels, the higher the value, the better the CE.

Figure 7 shows the performance of the proposed CE methods visually, by placing the sample images of each dataset along with their enhanced images using the proposed methods. In this figure, it can be seen that using the proposed methods, the contrast of the image has increased to a great extent and a large number of spots becomes visible. It can be seen that in method GA alone, in addition to the increase in the contrast of spots, the contrast of small artifacts is also increased and this can affect the accuracy of gridding and segmentation in the next steps. However, in the application of the GA + Denoising method, by reducing the artifacts, we can see a more accurate resolution of the spots, and the noises will cause much fewer errors in extracting the expression level of genes.

As mentioned earlier, the purpose of improving contrast is to improve image quality to increase the accuracy of MAI processing steps, i.e., gridding. Therefore, according to the results obtained from the previous section, the results of gridding on the enhanced images obtained with the proposed methods are compared to confirm the efficiency of CE. This step uses the proposed algorithm for gridding in section 2. Figure 8 shows the result of gridding for a randomly chosen sample image of the GMRCL dataset.

The gridding on the original image is unfitting due to its low contrast. On the other hand, gridding on enhanced images by GA has low accuracy due to background noise. This is while the GA + Denoising method provides accurate gridding, and the resolution of spots is even better when using the GA + Denoising method. However, gridding on the GA-enhanced image fails due to artifacts, which results in unrecognizable gridding.

To clarify this issue better, two poor-quality images are randomly selected from the datasets and the gridding algorithm is applied to them. The gridding results are shown in Figure 9. In this figure, the first column shows the Pheromone y744n32 subarray from the SMD dataset and the second column shows the 1313_ch1_OD730 subarray from the DeRisi dataset. Obviously, all the gridding results are unsuccessful in images without CE, but all of them

**Table 4: The best value of the fitness function in the genetic algorithm**

| Datasets | SMD | DERISI | GMRCL |
|---|---|---|---|
| Best fitness | 17.92 | 23.17 | 16.19 |

SMD – Stanford microarray database; GMRCL – Genomic medicine research center laboratory database; DERISI – Joe DeRisi's individual dataset

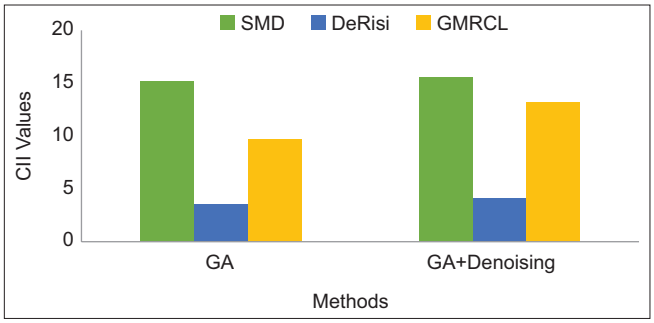


**Figure 6: Chart of the CII index values of the dataset's images with the proposed CE methods. AHE – Adaptive histogram equalization, CE – Contrast enhancement, GA – Genetic algorithm, SMD – Stanford Microarray Database, GMRCL – Genomic Medicine Research Center Laboratory**

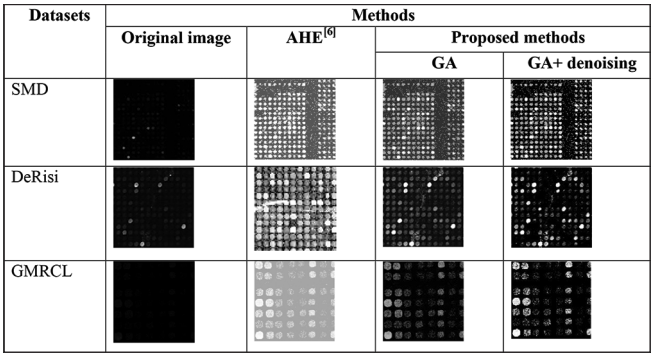


**Figure 7: Enhanced images of 3 datasets with the proposed contrast enhancement methods and comparison with adaptive histogram equalization method. GA – Genetic algorithm**

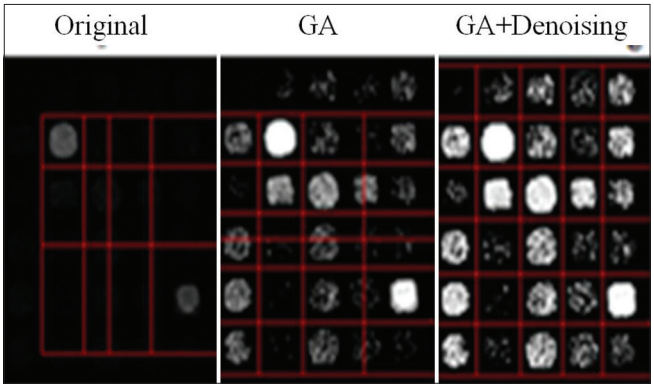


**Figure 8: Gridding on the original and enhanced images of the Genomic Medicine Research Center Laboratory dataset with the proposed methods. GA – Genetic algorithm**

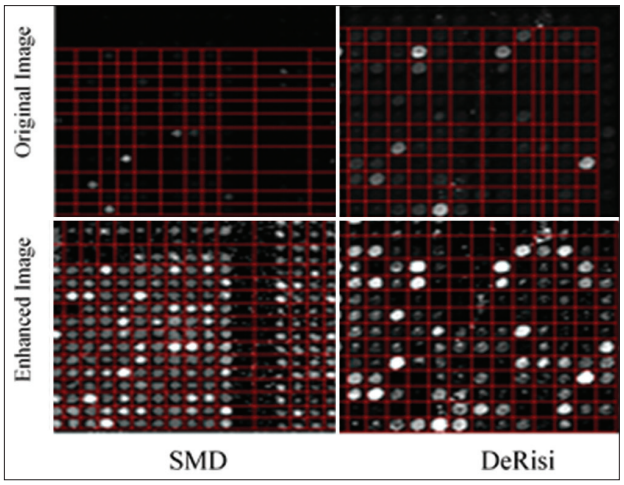


**Figure 9: The effect of contrast enhancement on gridding. First line: main image, second line: enhanced image with genetic algorithm + denoise2. SMD – Stanford Microarray Database**

are correct when applying the proposed CE method. For example, the correct grid lines for the image in the first column for contrast-enhanced mode are 15 horizontally and 14 vertically. However, for the mode without CE, 12 lines are horizontal and 10 lines are vertical, which means that several lines are lost. Furthermore, in the images of the second column, which are related to the DeRisi dataset, the effect of CE on the grid can be seen. Hence, before the CE, the spots and their positions are unclear, but after the CE, the spots and their location can be achieved regularly and are ready for segmentation. These experiments confirm that low image contrast greatly affects MAI gridding.

## Conclusion

Increasing image contrast is one of the most important preprocessing operations in image processing, and therefore, failure in this step leads to the shortcomings of subsequent image-processing steps. Furthermore, cDNA MAIs have a high priority among various image processing applications due to their great importance in cancer diagnosis. Some of the attractive methods for increasing the image contrast are metaheuristic methods, but so far, these methods have not been used to enhance the contrast of MAIs. Therefore, the main goal of the proposed method is to use the GA as a metaheuristic method to enhance the contrast of MAIs. In addition, in this paper, an efficient method for better image quality enhancement is proposed. In this method, one type of mathematical morphology was used to eliminate small artifacts as source noises, and their effect on contrast was investigated in combination with a GA. To evaluate the proposed methods, a total of 2 methods were implemented on 3 datasets of SMD, DeRisi, and GMRC. The results of the presented methods were evaluated with 6 evaluation indices ME, MLI, MI, CIR, CII, CPP, and the contrast value obtained by the method presented in the article. The proposed methods cause a significant increase in all indices, and by comparing the results for different datasets, a suitable method for improving the contrast can be chosen for each dataset. To show the effect of CE, images with and without CE were gridded, and the obtained results confirm the role of CE in the next stages of MAI processing.

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### Conflicts of interest

There are no conflicts of interest.

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