cDNA MICROARRAY IMAGING USING SINGLE-SENSOR TECHNOLOGY

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Abstract

A new solution for cDNA microarray image acquisition and processing is introduced. The proposed solution uses a two-color filter array placed on top of a single image sensor to capture the two-channel cDNA microarray data as a single monochromatic image, thus reducing twofold the memory and storage space. The vectorial nature of the cDNA microarray data is restored using the proposed image processing framework which also denoises and enhances the acquired image.

Keywords *— cDNA microarrays; single-sensor imaging; data acquisition; image denoising and enhancement.*

1 Introduction

Complementary Deoxyribonucleic Acid (cDNA) microarray imaging is a powerful technology used to extract and interpret genomic information in toxicological research, gene and drug discovery, and disease diagnosis [1]-[5]. The typical cDNA microarray formation process uses the well-known Cy3/Cy5 fluorescent system to produce two monochromatic images which are further registered into a two-channel, Red-Green image. The objective of the microarray experiment is to analyze the gene expression activity in the recorded samples.

Currently used microarray imaging systems operate either in a sequential or dual processing mode and produce two 16-bit monochromatic images for two (Cy3, Cy5) fluorescent dyes. The resultant two-channel image is stored in a tagged image file format (TIFF) for subsequent analysis. Depending on the array size and the scan resolution, the generated TIFF files often occupy between 30 and 120 MB per channel. Due to the nature of the acquisition process, acquired microarray images usually exhibit variations in intensity which affect the accuracy of the essential analysis procedures. Therefore, image processing operations, such as denoising/enhancement, data normalization, and spot localization, are routinely utilized to eliminate impairments and processing errors from propagating further down the processing pipeline to the gene expression analysis phase.

This paper introduces a new solution for cDNA microarray image acquisition and processing. The proposed solution uses a two-color filter array (CFA) placed on top of a single image sensor to capture the two-channel cDNA microarray data as a single monochromatic image. Thus, it reduces twofold the memory and storage space. Since the generated image has a mosaic layout due to the use of the CFA configuration, the two-channel cDNA microarray image is recovered from the acquired sensor data using demosaicking. The solution uses an adaptive edge-sensing mechanism and a spectral model to preserve the spatial and spectral characteristics of the demosaicked microarray images. Moreover, the proposed solution can also denoise and enhance the demosaicked image data.

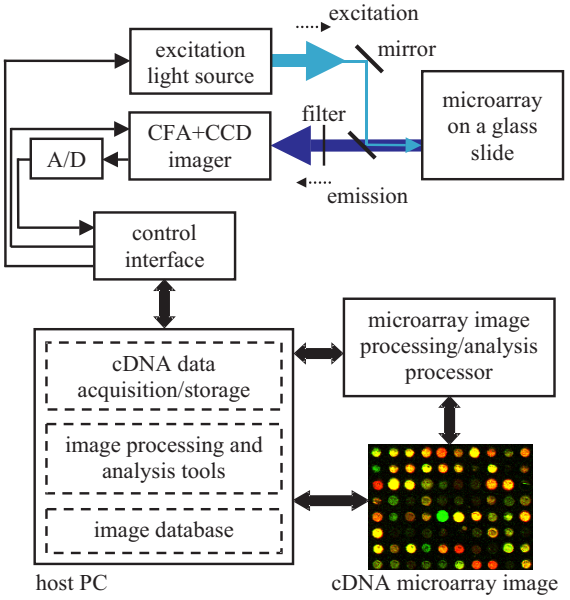

Figure 1. Proposed solution for cDNA microarray image acquisition, processing and analysis.

2 Single-Sensor cDNA Microarray Acquisition

Fig.1 shows the block scheme of the proposed solution. A multi-laser or white-light lamp is used to excite the (Cy3/Cy5) fluorophores in control (Cy3) and experimental (Cy5) cDNA samples. Emission optics collect the emitted fluorescence and direct it toward a detector which detects the emission radiation and converts it into voltage. Since Cy3 reaches peak absorption at 554 nm and emission at 568 nm while the corresponding wavelength for Cy5 are 650 nm for peak absorption and 672 nm for emission, two bandpass optical emission filters (i.e. a dichroic beam-splitter in con-

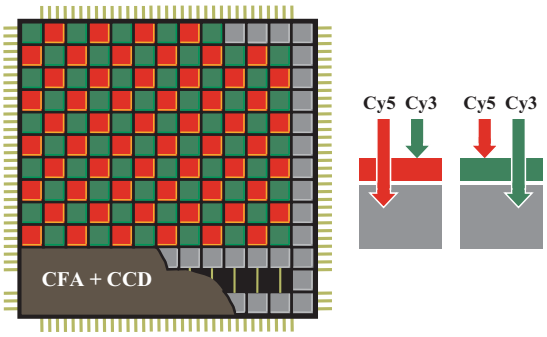

Figure 2. Proposed color filter array-based single-sensor configuration.

junction with a band-pass optical filter) are used to discriminate between excitation and emission photons and isolate the emission light with the corresponding wavelength, thus preventing distortion [4],[5].

In the proposed solution, a Red-Green (RG) color filter array (CFA) covers a single charge-coupled device (CCD) sensor (*Fig.2*). However, the approach can also be used in conjunction with a complementary metal oxide semiconductor (CMOS) sensor to develop an integrated on-chip microarray imaging/processing/analysis system. In the proposed CFA-CCD configuration, the CCD photodiodes with the R color (spectral) filter acquire the light emitted by the Cy5 fluorescent dye, while those covered by the G color filter acquire the photons emitted by the Cy3 dye. The wavelengths of the employed R and G filters correspond to the dominant wavelengths emitted by the Cy5 and Cy3 fluorescent dyes. Since the emitted signals corresponding to the experimental and control population are equally important to the cDNA image formation process, the CFA allocates 50% of sensor's spatial positions to Cy3 and the remaining positions to the Cy5-related intensities. The acquisition process generates a mosaic of Cy3 and Cy5-related values. After A/D conversion, the acquired data forms a $K_1 \times K_2$ single, mosaic-like, monochromatic microarray image z which consists of the scalar values $z_{(r,s)}$, for $r = 1, 2, ..., K_1$ and $s = 1, 2, ..., K_2$, corresponding to the signal intensities acquired for both Cy3 and Cy5 dyes.

3 cDNA Image Demosaicking

The two-channel cDNA microarray image $\mathbf{x}$ can be recovered from the monochromatic mosaic image z using the proposed demosaicking solution implemented in the supporting cDNA microarray image processor or running on a companion personal computer. Based on the CFA layout in *Fig.2*, z can be transformed to $\mathbf{x}$ using the R CFA components $x_{(r,s)1}$ located at the spatial locations $(r,s) \in \xi_1 = \{(\text{odd } r, \text{ even } s), (\text{even } r, \text{ odd } s)\}$, and the G CFA components $x_{(r,s)2}$ located at $(r,s) \in \xi_2 = \{(\text{odd } r, \text{ odd } s), (\text{even } r, \text{ even } s)\}$. In equivalent vector representation, pixels in $\mathbf{x}$ can be obtained from z as $\mathbf{x}_{(r,s)} = [z_{(r,s)}, 0]$ for $(r,s) \in \xi_1$ and $\mathbf{x}_{(r,s)} = [0, z_{(r,s)}]$ for $(r,s) \in \xi_2$ where zero values are used to indicate the missing components of the cDNA vector $\mathbf{x}_{(r,s)}$ located at (r,s).

The missing components $x_{(r,s)k}$, for $k = 1$ or $k = 2$, in vector $\mathbf{x}_{(r,s)}$ are obtained as follows [6],[7]:

$$x_{(r,s)k} = \sum_{(i,j)\in\zeta} w'_{(i,j)} x_{(i,j)k} = \frac{\sum\limits_{(i,j)\in\zeta} \{w_{(i,j)} x_{(i,j)k}\}}{\sum\limits_{(i,j)\in\zeta} w_{(i,j)}} \quad (1)$$

where $\zeta = \{(r-1,s), (r,s-1), (r,s+1), (r+1,s)\}$ denotes the spatial arrangement of the available components. In equation (1), the edge sensing weights [6],[7]:

$$w_{(i,j)} = \left\{ 1 + \sum_{(g,h)\in\varsigma} \left| x_{(i,j)k} - x_{(g,h)k} \right| \right\}^{-1} \quad (2)$$

are used to regulate the contributions of the available cDNA vectorial components $x_{(i,j)k}$ of the neighboring cDNA vectors $\mathbf{x}_{(i,j)} = [x_{(i,j)1}, x_{(i,j)2}]$, for $(i,j) \in \zeta$, by emphasizing cDNA inputs which are not positioned across a spot edge and directing the cDNA microarray demosaicking process along spot edges. Since a large value in the aggregated image gradient $\sum_{(g,h)\in\varsigma} \left| x_{(i,j)k} - x_{(g,h)k} \right|$ usually indicates that the corresponding component $x_{(i,j)k}$ is located across a spot edge, using weights inversely proportional to gradient values the corresponding small weight $w_{(i,j)}$ is used to penalize the associated input $x_{(i,j)k}$.

Demosaicking $x_{(r,s)1}$ using (1) with $k = 1$ at all locations listed in ξ_2 and $x_{(r,s)2}$ using (1) with $k = 2$ at all locations listed in ξ_1 produces a fully populated cDNA microarray image $\mathbf{x}$. To improve the accuracy of the demosaicked image $\mathbf{x}$, the demosaicked cDNA components can be re-evaluated by utilizing both spatial and spectral image characteristics as follows:

$$x_{(r,s)1} = x_{(r,s)2} + \sum_{(i,j)\in\zeta} w'_{(i,j)} (x_{(i,j)1} - x_{(i,j)2}) \quad (3)$$

$$x_{(r,s)2} = x_{(r,s)1} + \sum_{(i,j)\in\zeta} w'_{(i,j)} (x_{(i,j)2} - x_{(i,j)1}) \quad (4)$$

where $\zeta = \{(r-1,s), (r,s-1), (r,s+1), (r+1,s)\}$. The process uses spectral characteristics of the acquired cDNA data to reduce spectral artifacts and shifts in demosacked cDNA components. Spatial characteristics are used to preserve spot edges and ensure sharpness of the restored cDNA microarray image $\mathbf{x}$.

The above expressions have reasonable performance for various microarray data. However, in practice a number of impairments may affect the cDNA microarray image formation [8]-[10], making the normalizing cDNA component $x_{(r,s)2}$ in (3) or $x_{(r,s)1}$ in (4) usually deviating from the population of the neighboring cDNA components $x_{(i,j)2}$ in (3) or $x_{(i,j)1}$ in (4), respectively. Since the presence of outlying samples violates the assumption of significant spectral

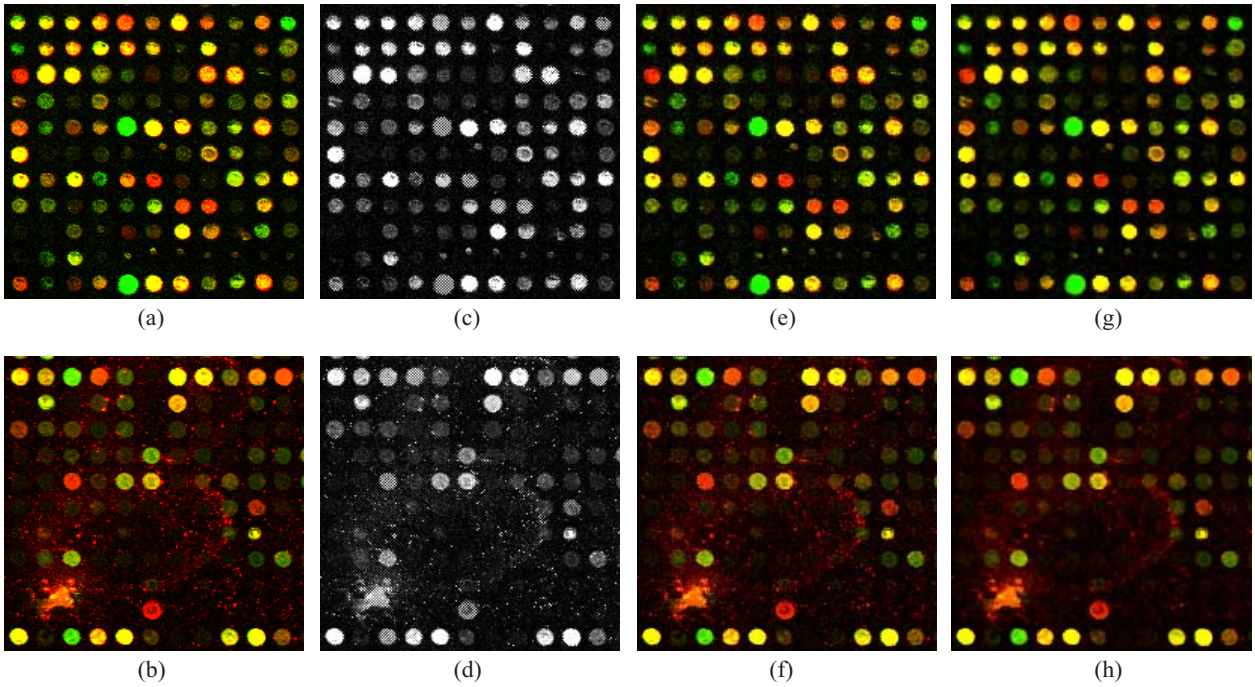

Figure 3. Achieved results: (a,b) cDNA images acquired using the conventional technology, (c,d) images acquired using the proposed solution, (e,f) demosaicked cDNA images, (g,h) demosaicked and denoised cDNA images.

correlations in (3)-(4), the use of an outlying sample in the normalization operations may produce intensity shifts and spectral artifacts. To make the proposed solution robust, equations (3)-(4) should be re-written as follows:

$$x_{(r,s)1} = \hat{x}_{(r,s)2} + \sum_{(i,j)\in\zeta} w'_{(i,j)}(x_{(i,j)1} - x_{(i,j)2}) \quad (5)$$

$$x_{(r,s)2} = \hat{x}_{(r,s)1} + \sum_{(i,j)\in\zeta} w'_{(i,j)}(x_{(i,j)2} - x_{(i,j)1}) \quad (6)$$

where $\hat{x}_{(r,s)2}$ and $\hat{x}_{(r,s)1}$ are robust estimates of the original input. In this work, we consider that $\hat{x}_{(r,s)2}$ and $\hat{x}_{(r,s)1}$ denote the output of the well-known median filter [11] calculated over the available cDNA components $\{x_{(r,s)2}, x_{(i,j)2}; (i,j) \in \zeta\}$ and $\{x_{(r,s)1}, x_{(i,j)1}; (i,j) \in \zeta\}$, respectively.

4 Experimental Results

The proposed cDNA microarray image processing solution was tested using real cDNA microarray images. These images were captured using conventional scanners. Examples with the spatial resolution of 200×200 pixels are shown in *Figs.3a,b*. To obtain the mosaic-like monochromatic images z shown in *Figs.3c,d* which are used as inputs to the proposed framework, the test images $\mathbf{o}$ were sampled using a RG CFA filter shown in *Fig.2* as follows: $z_{(r,s)} = o_{(r,s)1}$ for $(r,s) \in \xi_1$ and $z_{(r,s)} = o_{(r,s)2}$ for $(r,s) \in \xi_2$. Note that $o_{(r,s)k}$ denotes the R ($k = 1$) and G ($k = 2$) component of the cDNA vector $\mathbf{o}_{(r,s)} = [o_{(r,s)1}, o_{(r,s)2}]$ with $r = 1, 2, ..., 200$ and $s = 1, 2, ..., 200$.

Figs.3e,f show the demosaicked output obtained by utilizing processing steps (1) with $k = 1$ and $k = 2$ followed by (5) and (6). As it can be seen, the proposed adaptive processing framework preserves the structural content in the restored images. The reconstructed results follow both the structural content and the coloration of the conventionally acquired images. Moreover, if the demosaicked images are enhanced using (5) for $(r,s) \in \xi_1$ and (6) for $(r,s) \in \xi_2$, the framework normalizes cDNA image samples while removing foreground noise (see *Figs.3g,h* and for completeness also the cropped areas of the images shown in *Fig.4*). This suggests that the proposed framework can successfully perform a variety of on-chip integrated image processing steps.

5 Conclusions

A unique CFA-based framework for cDNA microarray image acquisition and processing was introduced. The proposed solution uses a bandpass emission filter system, a CCD megapixel sensor and an RG CFA to acquire simultaneously experimental and control cDNA channels and store the acquired data as a single, mosaic-like, monochromatic image. Demosaicking is used to restore the two-channel cDNA image from the acquired CFA microarray data. The same signal-processing solution can be also used to denoise and enhance the demosaicked cDNA microarray image.

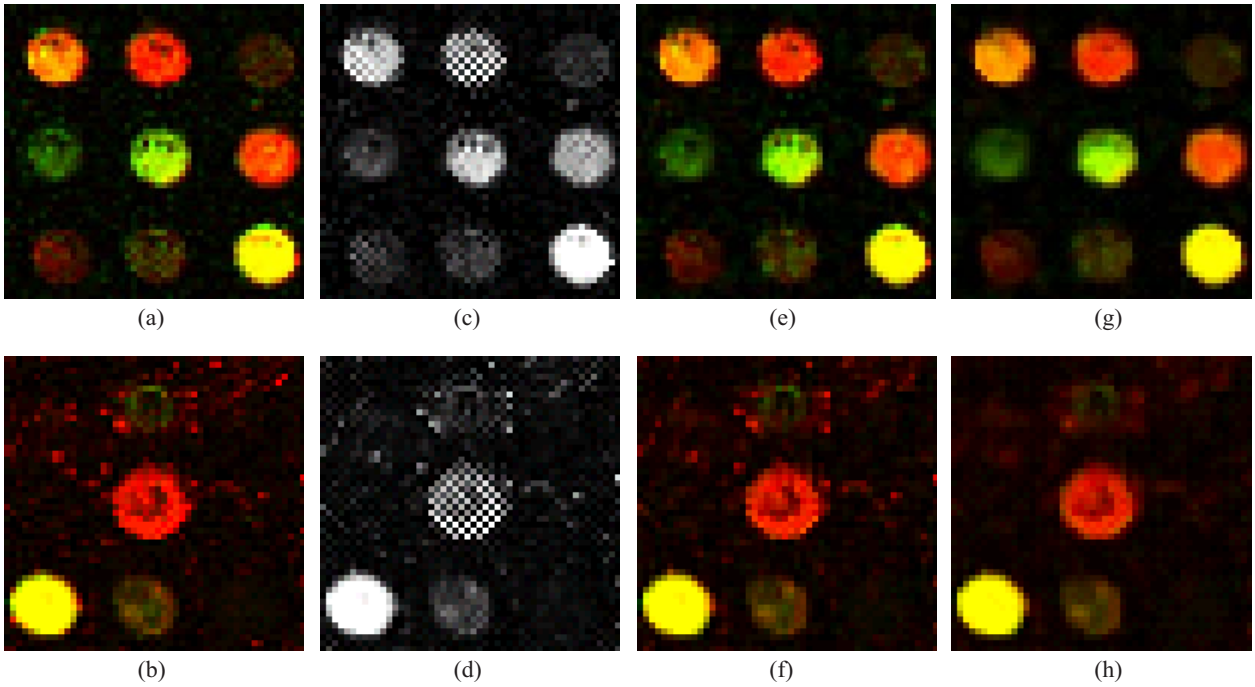

Figure 4. Cropped areas of the results shown in *Fig.3*: (a,b) cDNA images acquired using the conventional technology, (c,d) images acquired using the proposed solution, (e,f) demosaicked cDNA images, (g,h) demosaicked and denoised cDNA images.

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