Deconvolution of cDNA Microarray Images and Significance Testing for Gene Expression Levels

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1 Introduction.

The functional characterization and optimization of the microarray scanner are essential for the accurate quantitation of microarray data [1]. The most commonly used fluorescent dyes, Cy3 and Cy5, are relatively unstable, have different incorporation and quantum efficiencies, and are detected by the scanner with different efficiencies [2]. For these reasons, most microarray images are produced with different scanner settings, such as laser power and photomultiplier tube (PMT) gain between chips and between fluorescence dyes. However, there is neither a criterion for adjusting the scanner settings nor a proved relationship between the intensities of pixels in the microarray image and the 2-D fluorophore concentration (the quantity of fluorophores in unit area). Therefore, this manual adjustment must be checked for the accurate conversions of gene expression levels into the pixel intensities.

2 Software and files.

Several research papers indicate that the microarray scanner has dynamic range and that normalization between fluorescence labels is non-linear and depends on slides [3]. The relationship between fluorophore quantities and intensity reported by a scanner is linear only within a certain range of intensities, being dominated by noise below and subject to saturation above that range. There are many differences between the photochemical characteristics of both fluorescent tags. Therefore, the identical scanner settings for the Cy3 and Cy5 images do not convert the 2-D fluorophore concentration representing the gene expression levels into the images under the same condition.

Deconvolution is an image processing techniques, which is utilized for improving the contrast and resolution of digital images captured in the microscope. The synchronous deconvolution of both Cy3 and Cy5 images makes possible the reduction of the errors originated from scan process as well as the enhancement of the images. In a point of view that most gene expressions are *not* significantly up- nor down-regulated in cDNA microarray experiment, the spot intensity distributions of Cy3 and Cy5 images should be overlapped in a wide range. Thus, the synchronous deconvolution can make the equal prevalence of the spot intensities of Cy3 and Cy5 images as much as possible and increase the resolution of image intensity.

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