

Image Analysis and Measurement Quality

Imaging of the Cy3 and Cy5 dyes incorporated into the labeled cDNAs and localized by hybridization to the immobilized PCR products used as detector elements on the cDNA microarray slides was carried out using a confocal scanner equipped for dual laser excitation at the appropriate wavelengths. Placing one fluorescent image into the green channel and the other into the red channel formed pseudo-colored images. A detection method was then employed to determine the actual target region based on the information from both red and green pixel values [1]. The ratios of the red intensity to the green intensity R/G for all targets were determined, and then ratio normalization was performed based on 88 pre-selected internal control genes that are usually stable for most experiments (R/G ratio close to 1.0).

To further quantify the reliability of each ratio measurement, measurement of a set of quality indicators have been implemented in an improved version of the software program and applied in this study. An intensity measurement for either fluorescent channel is determined to be not reliable if one of the following conditions are not satisfied,

- 1) The average intensity measurement must be from a sufficiently large area,
- 2) The local background must be flat enough,
- 3) The signal consistency within the target area must be uniform enough, and
- 4) The majority of signal pixels should not be saturated.

For each ratio measurement, R/G , one further condition is imposed along with all of the four above conditions for each channel, that is, the average signal, $(R+G)/2$, must be strong enough (3x signal-to-noise ratio). Detailed discussion of the details of the quality implementation is in preparation for publication.

[1] Yidong Chen, Edward R. Dougherty, and Michael L. Bittner, Ratio-based decisions and the quantitative analysis of cDNA microarray images, *Biomedical Optics*, 2(4), p. 364-374, Oct. 1997.