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MICROARRAY IMAGE ENHANCEMENT TECHNIQUES USING THE DISCRETE WAVELET TRANSFORM

Emmanouil Athanasiadis[1], Dimitris Glotsos[1], Antonis Daskalakis[1], Panagiotis Bougioukos[1], Spyros Kostopoulos[1], Pantelis Theocharakis[1], Panagiota Spyridonos[1], Kalatzis Ioannis[2], George Nikiforidis[1] and Dionisis Cavouras[2]

[1] Medical Image Processing and Analysis Group, Laboratory of Medical Physics,
University of Patras, 26500 Patras, Greece.
e-mail: mathan@upatras.gr , web page: http://mipa.med.upatras.gr

[2] Medical Image and Signal Processing Laboratory, Department of Medical Instruments Technology,
Technological Institute of Athens, Greece.
e-mail: cavouras@teiath.gr , web page: http://medisp.bme.teiath.gr

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Abstract. *The objective of this work was to perform a comparative evaluation of five different wavelet-based filtering techniques in the task of microarray image denoising and enhancement. Clinical material comprised microarray images collected from the Oak Ridge National Laboratory. Image processing was performed in two stages: In the first stage an Exponential Histogram Equalization filter was applied in order to increase the contrast between spots and surrounding background. In the second stage, five wavelet-based image filters (Simple Piece-Wise Linear Mapping Filter (SPWLMF), Hard Threshold filter (HTF), Wavelet Enhancement with Noise Suppression filter (WEWNSF), Garrote Wavelet Threshold filter (GWTF) and Sigmoidal Non-linear Enhancement filter (SNLEF)) were implemented for denoising and enhancing gene microarray spots. The enhancing effectiveness of the five filters was assessed by calculating the Mean-Square-Error (MSE) and the Signal-to-MSE ratio. Results showed that the image quality of the processed images was superior to that of the original images. Significant noise suppression was accomplished by the SPWLMP filter, which scored the minimum MSE and the maximum Signal-to-MSE ratio. Processing time was less than 3 seconds for 512x512 sample images. Wavelet-based processing of microarray images was found to enhance microarray images effectively, by improving the visualization of spots and by suppressing image noise.*

1 INTRODUCTION

Image processing is an area that wavelet-based techniques have proven to perform successfully. Microarray imaging is considered to be an important tool in bioinformatics. The main benefit of this technique is that it can observe thousands of genes simultaneously. The identification of the genes is closely related with the identification of spots. Due to various sources of noise during image processing [1], the outline of each spot is irregular and, thus, the mean intensity measurements are not accurate [2]. Additionally, the location of the arrayer, as well as sub-arrays contained within the main grid, may vary from image to image. This is due to imperfections during the construction of the arrayer. Moreover, contamination could affect measurements during the scanning procedure [3].

Several methods have been introduced in the past. Statistical methods that include analysis of variance have been introduced by Kerr [4], ratio distribution by Chen [5] and Ermolaeva [6], and Gamma distribution by Newton [7]. All these methods deal with measurement errors, such as cross hybridization. However, the effect of noise has not been previously dealt with.

In the present study, a systematic evaluation of five wavelet-based noise suppression filters was performed regarding the enhancement of microarray images.

2 MATERIALS AND METHODS

2.1 Material

On complementary DNA (cDNA) Microarray experiments [1], two messenger RNA (mRNA) samples are first reverse transcripted into cDNA. These two samples are labeled using two different fluorescence dyers, Cyanine Cy3 (red channel) and Cy5 (green channel) respectively. The samples are next hybridized [3] and following the scanning procedure, two colored fluorescence Tagged Image File Format (TIFF) images are produced for each channel [3]. The fluorescence intensity value of each spot is related to the expression abundance of the corresponding DNA sequence. In the present study, a dataset of 32, 8-bit grayscale TIFF microarray images (16 for the Red Cy3 channel and 16 for the Green Cy5 channel) were collected from the public database of *the Oak Ridge National Laboratory* [8].

2.2 Histogram equalization filter

The histogram equalization algorithm, using exponential mapping functions, was developed and applied to images [9, 10, 11]. The histogram equalization function is based on the corresponding probability density models for the desired exponential distribution output graylevel histogram. Equation 1 provides the output probability density model and equation 2 the corresponding mapping function.

$$P_g(g) = c \cdot \exp[-c(g - g_{\min})] \tag{1}$$

$$g = g_{\min} - \frac{1}{c}\ln(1 - CDF) \tag{2}$$

where, g_{min} is the minimum graylevel in the image, *CDF* is the Cumulative Distribution Function of the histogram and *g* is the calculated gray-tone of the processed image.

2.3 Wavelet-based filters

The wavelet-based enhancement involved three steps [12, 13, 14, 15, 16]. First, the DAUB4 DWT (Discrete Wavelet Transform) [12] was applied in two scales for each microarray. Second, the detail coefficients were processed in both scales, using in each occasion one of the five different enhancement functions given in sections 2.3.1 to 2.3.5. Finally, the processed images were reconstructed using the Inverse DWT (IDWT) [12]. Schematically, the overall process is described in Figure 1.

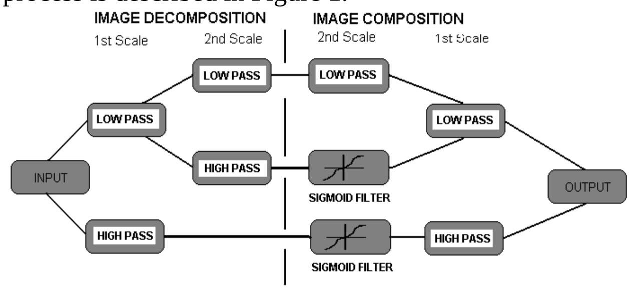


Figure 1. Wavelet-based filtering scheme

2.3.1 Simple Piece-Wise Linear Mapping (SPWLMF) Function.

In the SPWLMF filter, the multi-scale coefficients of the DWT were modified according to equation 3. An appropriate threshold value |*T*| as well as an amplification factor *G* were manually chosen for optimal results. Schematically, the redistribution of the wavelet coefficients is illustrated in figure 2.

$$W_{out} = \begin{Bmatrix} W_{in} + T \cdot (G-1) \text{ if } W_{in} > T \\ W_{in} - T \cdot (G-1) \text{ if } W_{in} < -T \\ G \cdot W_{in} \quad \text{otherwise} \end{Bmatrix} \tag{3}$$

were *Wout* denotes the output and *Win* the input coefficient values of the Detail Matrix. *T* and *G* are threshold and gain values respectively.

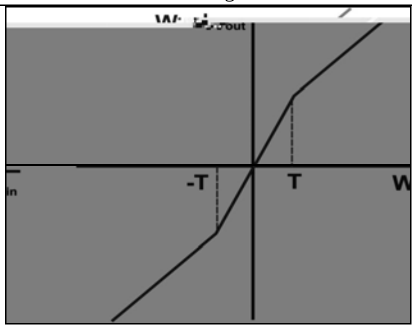


Figure 2: Simple Piece-Wise Linear Mapping Function (*F1*)

2.3.2 Hard-Threshold Function (HTF) Function.

In the HTF filter, the modification process of the wavelet coefficients was similar to SPWLMF. The major difference was that the values between the thresholds |*T*| were neutralized. HTF is illustrated in equation 4 and figure 3 respectively.

$$W_{out} = \begin{cases} W_{in} + T \cdot (G-1) \text{ if } W_{in} > T \\ W_{in} - T \cdot (G-1) \text{ if } W_{in} < -T \\ 0 \quad \text{otherwise} \end{cases} \tag{4}$$

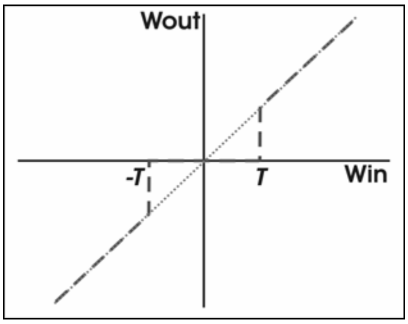


Figure 3: Hard-Threshold Function unction (*F2*)

2.3.3 Wavelet Enhancement With Noise Suppression (WEWNS) Function.

According to the WEWNS filter, two independent threshold values, $|T_1|$and $|T_2|$, were selected and the wavelet coefficients were processed using equation 5. WEWNS is also illustrated schematically by Figure 4.

$$W_{out} = \begin{cases} W_{in} + T_2 \cdot (G-1) - T_1 \cdot G & \text{if } W_{in} > T_2 \\ G \cdot (W_{in} - T_1) & \text{if } T_1 < W_{in} \le T_2 \\ 0 & \text{if } -T_1 \le W_{in} \le T_1 \\ G \cdot (W_{in} + T_1) & \text{if } -T_2 \le W_{in} < -T_1 \\ W_{in} - T_2 \cdot (G-1) + T_1 \cdot G & \text{if } W_{in} < -T_2 \end{cases} \tag{5}$$

were, *Wout* denotes the output and *Win* the input coefficient values of the detail wavelet coefficient matrix, *G* is the gain value, while T_1 and T_2 are two threshold values[3,4].

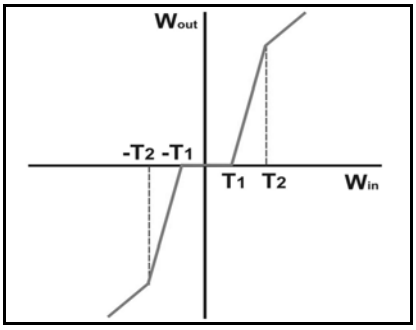


Figure 4: Wavelet Enhancement With Noise Suppression function (*F3*)

2.3.4 Garrote Enhancement (GEF) Function.

Regarding the GEF filter, the multi-scale coefficients of the DWT were modified according to equation 6. GEF is also illustrated schematically in Figure 6.

$$W_{out} = \begin{cases} W_{in} - \dfrac{T^2}{W_{in}} \text{ if } |W_{in}| > T \\ 0 \quad \text{otherwise} \end{cases} \qquad (6)$$

where W_{out} denotes the output coefficients, W_{in} are the input coefficients and parameters T and G control the threshold and the gain respectively.

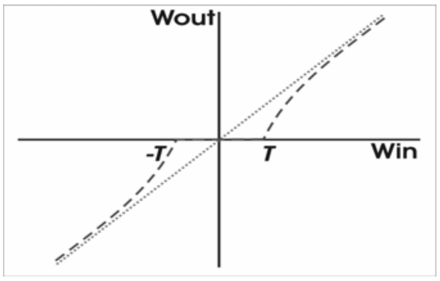


Figure 5: Garriote Enhancement Function (*F4*)

2.3.5 Non Linear Enhancement (NLEF) Function.

According to the NLEF filter, wavelet coefficients between the threshold values |*T*| were squared, as illustrated in equation 5 and figure 6.

$$W_{out} = \begin{cases} W_{in} + T \cdot (1-T) \text{ if } W_{in} > T \\ W_{in} - T \cdot (1-T) \text{ if } W_{in} < -T \\ {W_{in}}^2 \quad \text{otherwise} \end{cases} \qquad (7)$$

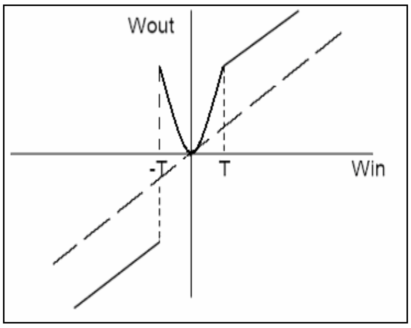


Figure 6: Non Linear Enhancement Function (*F5*)

2.4 Evaluation.

In order to provide a quantitative measure of the performance of each algorithm, the mean-square-error (MSE) and Signal-to-MSE (S/MSE) ratio were calculated [17]. MSE and S/MSE were defined by equations 8 and 9 and were calculated over a local region-of-interest (ROI).

$$MSE = \frac{1}{k}\sum_{i=1}^{k}(\overline{S_i} - S_i)^2 \qquad (8)$$

$$\frac{S}{MSE} = 10 \times \log_{10}\left(\frac{\sum_{i=1}^{k} S_i^2}{\sum_{i=1}^{k}(\overline{S_i} - S_i)^2}\right) \qquad (9)$$

where S is considered to be the original image, $\overline{S_i}$ the processed image, and K is the total number of pixels within the current ROI.

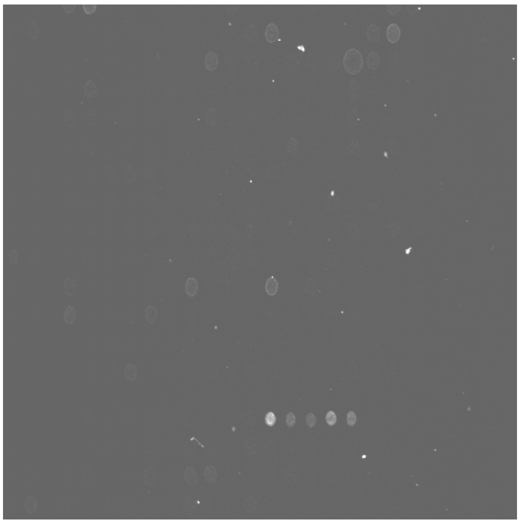
3 RESULTS AND DISCUSSION

According to our results, the SPWLMF filter was found to suppress more effectively image noise. It was also observed that the SPWLMF filter achieved the lowest and the highest MSE and S/MSE respectively in all of the cases. Table 1 illustrates a typical example of MSE and S/MSE measurements for one ROI.

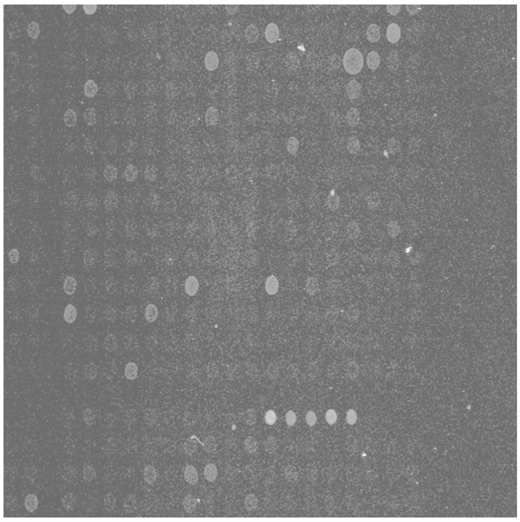
Filters	**MSE**	**S/MSE**
SPWLMF (F1)	9,13	17,54
HTF (F2)	9,15	17,52
WEWNS (F3)	9,15	17,52
GEF (F4)	12,76	16,09
NLEF (F5)	9,15	17,52

Table 1. ROI MSE and S/MSE measurements of 5 different wavelet-based filters.

Figure 7 shows the initial Microarray image as well as the processed by the histogram equalization filter. Moreover, the histogram equalized image, processed by SPWLMF algorithm, is also illustrated in Figure 8.



(a)



(b)

Figure 7: (a) Initial Microarray image and (b) processed with histogram equalization

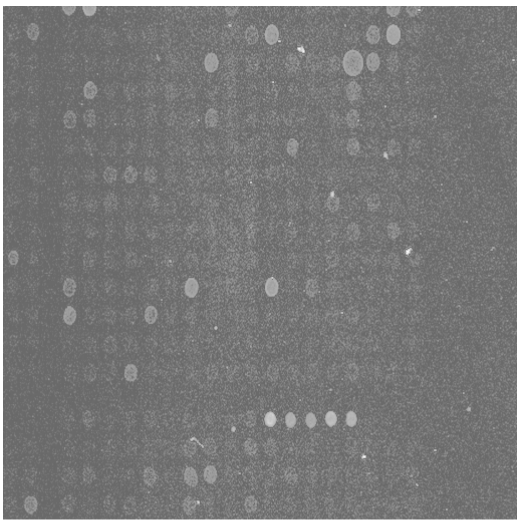


Figure 8: Result of SPWLMF wavelet-based noise suppression filter.

4 CONCLUSIONS

In accordance with our findings, the performance of the SPWLMF filter was superior to that of the other wavelet based filters. Processing time was less than 3 seconds for the 512x512 sample images. Wavelet-based processing of microarray images was found to enhance images effectively, by improving the spots' visualization.

5 ACKNOWLEDGEMENTS

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6 REFERENCES

[1] Y.H. Yang, M. J. Buckley, S. Duboit and T.P.Speed (2002), "Comparison of methods for Image Analysis on cDNA Microarray Data", Journal of Computational and Graphical Statistics, vol. 11, pp 108-136.

[2] K. Blekas, N.P. Galatsanos and I. Georgiou (2003), "*An unsupervised Artifact Correction Approach for the Analysis of DNA Microarray Images*", in Proc. IEEE International Conf. on Image Processing (ICIP), vol 2, pp 165-168.

[3] Y. Wang, F.Y. Shih, M.Q. Ma.(2005) "*Precise Gridding of Microarray Images By Detecting and Correctiong Rotation in Subarrays*". Sixth Inter. Conf. on Computer Vision, Pattern Recognition and Image Processing, Salt Lake City, UT, July.

[4] M. K. Kerr, M. Martin, and G. A. Churchill (2001), "Analysis of variance for gene expression Microarray data", J. Comput. Biol., vol. 7, pp. 819 -837.

[5] Y. Chen, E. R. Dougherty, and M. L. Bittner (1997), "Ratio- based decision and the quantitative analysis of DNA Microarray images", J. Biomed. Opticcs. pp 364-375.

[6] O. Ermolaeva, M. Rastogi, K. D. Pruitt, G. D. Schuler, M. L. Bittner, Y. Chen, R. Simon, P. Meltzer, J.M. Trent and M. Boguski (1998),"Data management and analysis for gene expression arrays", Nature Genetics, vol.20, pp. 19-23.

[7] M.A. Newton, C.M. Kendziorski, C. S. Richmond, F. R. Blattner, and K. W. Tsui,(2001) "On differential variability of expression ratios: Improving statistical inference about gene expression changes from Microarray data", J. Computat. Biol. , vol. 8, pp. 37-52.

[8] http://homer.hsr.ornl.gov/CBPS/

[9] Pratt W. K. (1991): *Digital Image Processing (Second Edition)*, John Wiley & Sons, New York, pp. 275-285.

[10] Gonzales R. C., Woods R. E. (1992): *Digital Image Processing*, Addison-Wesley, pp. 167-190.

[11] Weeks A. A. (1996): *Fundamentals of Electronic Image Processing*, Wiley-IEEE Press, pp. 109-120.

[12] Daubechies I. (1988): "Orthonormal Bases of Compactly Supported Wavelets", Communication of Pure and Applied Math. 41, pp. 909-996.

[13] Mekle R, Laine A., Smith S., Singer C., Koenigsberg T., Brown M.(2000): "*Evaluation of a multiscale enhancement protocol for digital mammography*", Wavelet Applications in Signal and Image Processing VIII, Akram Aldroubi; Andrew F. Laine; Michael A. Unser; Eds. Proc. SPIE 4119, pp. 1038-1049.

[14] Brown T. J. (2000): "*An adaptive strategy for wavelet based image enhancement*", Proceeding of IMVIP 2000 - Irish Machine Vision and Image Processing Conference, Dublin, Ireland, pp. 67-81.

[15] Brown T. J. (2001): "*Differential Image Enhancement via Wavelet Foveation*", Proceeding of IMVIP 2001-

Irish Machine Vision and Image Processing Conference, Dublin, Ireland, pp. 179-192.

[16] E. Athanasiadis, N. Piliouras, K. Sidiropoulos, H. Georgiou, C. Makris, N. Dimitropoulos, D. Cavouras (2004), "*Mammographic Image Enhancement Using Wavelet-Based Processing And Histogram Equalization*", 1st International Conference "From Scientific Computing to Computational Engineering" (1st IC-SCCE), Athens, Greece, September 10-18.

[17] Konstantinos P. Sidiropoulos, Nikos G. Piliouras, Emmanouil I. Athanasiadis, Harris V. Georgiou, Christos S. Makris, Nikos Dimitropoulos, Dionisis A. Cavouras (2004), "*Statistical Versus Wavelet-Based despeckling techniques for enhancing medical ultrasound images*", 1st International Conference "From Scientific Computing to Computational Engineering" (1st IC-SCCE), Athens, Greece, September 10-18.