Segmentation of Two Dimensional Electrophoresis Gel Image Using the Wavelet Transform and the Watershed Transform

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Abstract— **Segmentation of two dimensional electrophoresis (2DE) gel image is a challenging task due to presence of non-linear backgrounds, horizontal and vertical streaks, and irregular spots. The watershed method is a powerful tool for medical image segmentation, but it produces over-segmented results due to presence of noise and non-linearity. The solutions available in literature have failed to give satisfactory results in case of gel images. This paper presents a novel method for segmentation of 2DE gel images. The pitfalls of the watershed transform have been addressed through spot characterization in the wavelet domain. The wavelet transform is an important multi-scale analysis tool for the images. The proposed method utilizes the best features of both the watershed and the wavelet transforms in which connected maxima set corresponding to each watershed region has been introduced and computed in the wavelet domain. This allows us for accurate detection of the spots in each watershed region. Experimental results on the set of real gel images demonstrate that our method outperforms the commercialized software. Our method has also an advantage of single threshold parameter selection.**

***Index Terms*— Gel image, watershed, quincunx, wavelet, spot charactrization, connected maxima set.**

I. INTRODUCTION

Two dimensional electrophoresis is an important technique for analyzing protein expression and for enhancing data quality in the field of proteomics. Proteomics is the field that studies a multi-protein system, focusing on the interplay of multiple proteins as functional components in a biological system. By this technique, a very large number of proteins can easily and simultaneously be separated, identified and characterized. This is important for understanding protein function and thus enables the development of new and more effective drugs [1].

The first step in a typical proteomics analysis workflow is protein separation, followed by quantification and differential expression analysis. Despite its limitations, 2D gel electrophoresis (2DGE) remains the most widely used protein separation method. Using 2DGE, individual proteins in a mixture are resolved in the first gel dimension according to their molecular weight and in the second dimension according to their isoelectric point [2][3].

A very important task in a proteomics study is the correct analysis and interpretation of 2D gel images through image analysis. A typical gel image is shown in Fig. 1. The objective is to extract the protein spots in gel images from the uneven background which has sharp edges, e.g. lines, artifacts and streaks in some area. Due to some technical problems such as the system nonlinearities in gel formation and image acquisition, inevitably there appear overlapped protein spots, saturated spots, faint spots, nonlinear intensity and narrow lines on the gels which make the task more difficult [3][4]. Several methods [5][6][7] have been referred to in the literature, they all can be seen as variants of the watershed method and their results depend very much upon the sensitivity of parameter selections and post-processing thereafter.

In this paper, a novel method for segmentation of gel images has been presented. First, the watershed transform and quincunx wavelet transform are applied on the image and then spot characteristics have been formulated through a set of rules. Our approach seems to outperform the commercialized software, for which we have presented some results.

This paper is organized as follows. Section 2 gives an overview of the watershed transform and the methods available in literature to reduce its over-segmentation problem. Section 3

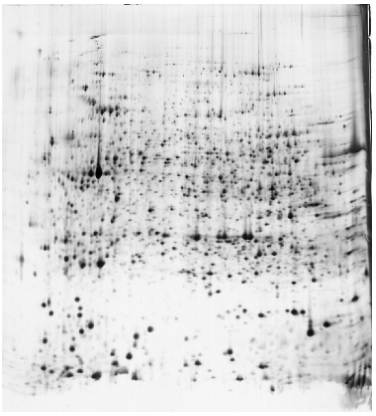

Figure 1. A Typical 2DE Gel Image

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discusses the quincunx wavelet transform, and shows why and how it is used in our method. Section 4 presents our novel method of spot characterization in the watershed and the wavelet domain. Section 5 demonstrates the experimental results on real gel images as well as comparison with Delta 2D, a well-known renowned commercialized software package for image analysis of 2DE gel images. Finally, conclusion and future perspective of our work are discussed in section 6.

II. THE WATERSHED TRANSFORM

The watershed transform [8] is a powerful tool for medical image segmentation. It is a region growing algorithm that analyzes an image as a topographic surface. The grey level 'h' of a pixel (x, y) of the image becomes the elevation 'h' of a point (x, y, h). In this way, the image surface can be seen as full of mountains and valleys. Let us pierce a hole in all local minima and immerse it into a lake. Water will fill up the valley starting at these local minima, and at all points where waters coming from two or more valleys will meet, dams are built. As a result, the surface is partitioned into different regions separated by dams. The regions so formed are called catchment basins and the dams are called watershed lines. Numerous techniques have been proposed to compute the watershed [9][10]. The advantage of the watershed approach is that it produces closed, adjacent and accurate contours. However, the watershed transform often leads to over-segmentation. There are two main approaches in the literature to overcome this problem. The first is region merging [11], i.e. merge the adjacent region according to some similarity criteria. Images containing large and nonlinear variations in intensities of pixels often lead to poor results due to the difficulty of choosing optimized and varying similarity criteria. Gel images are typical examples of non-stationary signals and the region merging approach does not yield satisfactory results. Another approach to overcome over-segmentation is use of markers [12]. A marker is a connected component on an image. Internal markers are associated with the objects of interest and external markers are associated with the background. These markers are imposed as minima on the image and all other minima are suppressed. Then the watershed algorithm is applied on the image. The disadvantage of this method is that the accuracy of the result depends upon the accurate placements of markers and number of markers. For the gel image containing irregular spots with varying intensities, it is mostly difficult to find out accurate markers.

III. THE QUINCUNX WAVELETTRANSFORM

The wavelet transform [13] decomposes the signal in terms of a family of functions generated from a prototype function, called the mother wavelet, by dilation and translation operations. The quincunx wavelet transform is the simplest two- dimensional non-separable decomposition. It uses non-separable and non-oriented filters followed by the non-separable sampling as represented by the following matrix [14]:

$$Dq = \begin{bmatrix} 1 & 1 \\ 1 & -1 \end{bmatrix} \qquad (1)$$

The determinant of the matrix Dq has an absolute value of two, and therefore, the corresponding critically sampled filter bank will have two channels. Furthermore, the input image is decomposed with the multiresolution scale factor of $\sqrt{2}$, resulting in one low-resolution subimage and one non-oriented wavelet subimage. The quincunx wavelet transform is more isotropic than separable 2D wavelet transform and yields fewer artifacts [15]. That is why we prefer to use the quincunx wavelet transform. The undecimated version of the transform is used since the same size of image is obtained at each scale and it is easy to map watershed regions on it. The desirable properties of the quincunx wavelet are perfect reconstruction (PR), linear phase, good frequency selectivity, and satisfactory vanishing moments. Designing non-separable filter banks with all these properties is a challenging task. Therefore, instead of using the standard canonical polyphase structure, the lifting factorization [16] is often more convenient to design and implement the Quincunx filter bank. The lifting structure guarantees PR, and the so-called predict and update lifting steps can be used to increase the order of the polyphase matrix (and thus of the filters) while maintaining PR. We have used quincunx interpolating filter banks having 4 primal and 4 dual vanishing moments, designed by Kovacevic and Sweldens [17], and based on the lifting scheme [16]. We have found that using 4 primal and 4 dual vanishing moments, results are better than using 2 primal and 2 dual vanishing moments. For the undecimated version, we remove the decimation operator from the quincunx lifting scheme [17] and the N-times quincunx upsampled versions of predict and update filters are used, where N represents decomposition level. This way for higher decomposition levels, the corresponding filter support widens.

IV. SPOT CHARACTRIZATION AND SEGMENTATION

Spot characteristics have been analyzed in the watershed and wavelet domains. First, a watershed transform is applied on the low resolution image of the same size and region information is imposed on to the original image. A low resolution image is used to reduce over-segmentation, which is also beneficial in reducing processing requirements. The resolution should not be too low as it causes a problem in the spot detection. The image is segmented into homogeneous regions called catchment basins (CB). Let $\{m_k\}$ be the minima of image I and $\{CB(m_k)\}$ be the associated catchment basins. Each catchment basin may contain spots as well as a background. We define two subsets within catchment basins as follows:

Definition 1: Let F and B be two subsets in a catchment basin $CB(m_k)$ such that $F \cap B = \emptyset$. We say F is a set of pixels belonging to spots of image I, if

(1) For any $(x_f, y_f) \in F$ and any $(x_b, y_b) \in B$,

$$I(x_f, y_f) < I(x_b, y_b).$$

where I(x, y) denotes the grey value of pixel p (x, y) in image I.

(2) There exists an optimal 'h', such that threshold of I at level h is defined as

$$T_h(I) = \{p | I(p) < h\}$$

and $$F = CB(m_k) \cap T_h(I)$$

It is clear from definition 1 that to distinguish F from other parts of the image I, we need to find out the optimum value of h.

Image I is further decomposed using the quincunx wavelet transform and detail coefficients Wj at scale j are given by

$$W_j(x,y) = I * \psi_j(x,y)$$

where ψ_j is a wavelet at scale j and * denotes the convolution.

To de-correlate the noise across wavelet scales, we introduce the term "scale product" as follows:

Definition 2: The scale product $P_{j,j+1}$ shows the correlation between wavelet coefficients of adjacent scales j and j+1 and defined as

$$P_{j,j+1}(x,y) = W_j(x,y).W_{j+1}(x,y)$$

Watershed catchment basins are mapped onto $P_{j,j+1}$ and corresponding to each $CB(m_k)$, coefficients of $P_{j,j+1}$ are found and denoted as $S_{j,j+1}(m_k)$. Now a connected maxima set for $S_{j,j+1}(m_k)$ is defined as follows:

Definition 3: A connected maxima set $CM_i(m_k)$ is a collection of those coefficient $\{c_q\text{'s}\}$ of $S_{j,j+1}(m_k)$ which satisfies any of the following criteria:

(1) $c_q = \{\max_j(c_j) | c_j \in \left(S_{j,j+1}(m_k) - \cup_{l\neq i} CM_l(m_k)\right)\}$

or

(2) c_q is in neighborhood of $CM_i(m_k)$ and it is largest of its neighbors. Also c_q has at least (k-2) neighbors having value less than it (assuming k-neighborhood operation).

In a $S_{j,j+1}(m_k)$, there may be more than one connected maxima set. Horizontal and vertical connected maxima sets are identified using the following definition.

Definition 4: A connected maxima set $CM_i(m_k)$ is a horizontal connected maxima set $HCM_i(m_k)$ if $\exists \epsilon > 0\ such\ that\ \forall c_q \in CM_i(m_k)$ and $\forall c_q \in CM_i(m_k), |y_{c_q} - y_{c_p}| < \epsilon$. Similarly, a connected maxima set is a vertical connected maxima set $VCM_i(m_k)$ if $\exists \epsilon > 0\ such\ that\ \forall c_q \in CM_i(m_k)$ and $\forall c_q \in CM_i(m_k), |x_{c_q} - x_{c_p}| < \epsilon$, where (x_{c_q}, y_{c_q}) is coordinate of c_q in $P_{j,j+1}$. (We are taking $\epsilon = 1$ in our experiments.)

The vertical and horizontal connected maxima sets are less likely to be the features of a spot, therefore these are neglected and the union of the connected maxima set is defined as follows:

$$UCM(m_k) = \cup_{\forall i} \{CM_i(m_k) | \forall j, CM_i(m_k) \neq HCM_j(m_k) \text{ and } CM_i(m_k) \neq VCM_j(m_k)\}$$

The coefficient c_{max} which has a maximum value among the coefficients of all connected maxima sets in $UCM(m_k)$ is selected.

$$c_{max} = \max\{c_q | c_q \in UCM(m_k)\}$$

Let us denote the coordinates of c_{max} in $P_{j,j+1}$ as $(x_{c_{max}}, y_{c_{max}})$. Now, the threshold of I at level 'h' introduced in definition 1 can be found out. A simple strategy is used for determining optimal value of 'h' and it gives the accurate results. The strategy is stated as follows:

$$h = \{I(x,y) | I(x,y) \in CB(m_k), x = x_{c_{max}}, y = y_{c_{max}}\}$$

After this, pixels can be clearly classified as a member of either F or B.

For any subset $X \subseteq CB(m_k)$ in the image I, the corresponding scale product coefficients Coeff(X) is found as:

$$Coeff(X) = \{c_q | c_q \in S_{j,j+1}(m_k),\ c_q = P_{j,j+1}(x_{c_q}, y_{c_q}) \text{ and } I(x_{c_q}, y_{c_q}) \in X\}$$

where (x_{c_q}, y_{c_q}) is the coordinate of the coefficient c_q in the scale product $P_{j,j+1}$.

The mean M(X) and the k-th order center moment $\mu_k(X)$ of the wavelet coefficients corresponding to a subset $X \subseteq CB(m_k)$ is calculated as follows:

$$M(X) = \frac{1}{numel(X)}[\sum_i (Coeff_i(X)]$$

$$\mu_k(X) = \frac{1}{numel(X)}\left[\sum_i (Coeff_i(X) - M(X))^k\right]$$

where numel(X) is the number of elements in the subset X and $Coeff_i(X)$ is the i^{th} element of the set Coeff(X).

To distinguish between artifacts and spots, a simple criterion based on the second and the third order center moments, has been formulated. According to this, if the second and/or the third order moments of F are more distinguishable than that of B, then F can be considered as a true spot. The criterion has been successfully used in our experiments and can be stated as follows:

$$Dist(F,B) = \sqrt{\left[\left(\mu_2(F) - \mu_2(B)\right)^2 + \left(\mu_3(F) - \mu_3(B)\right)^2\right]}$$

For a catchment basin $CB(m_k)$, if $Dist(F,B) > T$, then F is considered as a spot, otherwise it is considered as an artifact (where T is a single threshold used for each catchment basin).

V. Methodology and Experimental Results

Our methodology is depicted in Fig. 2. The image is first de-noised using the quincunx wavelet transform [18]. Then again the quincunx wavelet transform is applied to the de-noised image up to the decomposition level J=3. The low resolution image at scale J is used for watershed segmentation and detail components of scale 2 and 3 are used for computing the scale product. The post-processing step contains morphological operations (erosion followed by dilation using disk shaped structure) to remove remaining streaks and to improve the results.

8-bit gray level images of size 1024 x 1024 and of size 512 x 512 have been used. The segmentation that we have achieved is clearly more accurate than that of the popular commercialized software Delta 2D. The segmentation result of a gel image is shown in Fig. 3. The results of commercialized

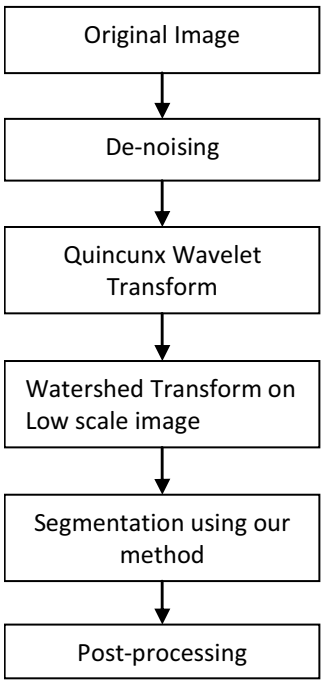


Figure 2. Our methodology

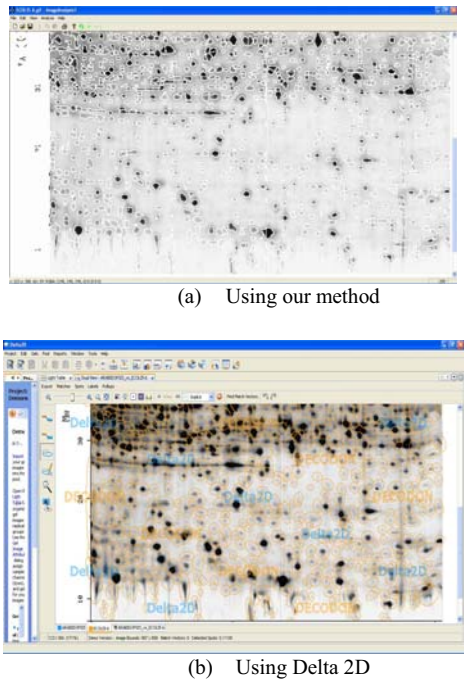
software including Delta 2D depends upon three or more sensitivity parameters. As an advantage our method uses a single threshold parameter. This method is also employed to detect faint spots but artifacts are problems as they are in case of commercialized software. The number of artifacts (false spots) and missing spots have been found less as compared to Delta 2D. Missing spots include the spots that are not detected at all or that are detected as a part of other spots. False spots are the artifacts detected by the software since they pass all conditions of the software to prove themselves as spots. In Fig. 4, a part of the gel image has been shown with missing spots and false spots. To compare our method with Delta 2D, we define the 'spot efficiency' factor as follows:

$$\text{Spot efficiency} = \frac{\text{Total spots detected} - \text{No.of False spots}}{\text{Total spots detected} + \text{No.of Missed spots}}$$

Clearly, if the number of false spots is more, the spot efficiency will be less and if the number of missing spots is more, spot efficiency again will be less. For a method, spot efficiency will be 1 (100%) if and only if no false spot is detected and no real spot is missed. Table I summarizes the results using our method and using Delta 2D.

TABLE I. RESULTS IN TERMS OF 'SPOT EFFICIENCY'

	Our Method	**Delta 2D**
Image1	96%	94%
Image2	94%	91%
Image3	96%	93%
Image4	91%	87%
Image5	97%	95%



(a) Using our method

(b) Using Delta 2D

Figure 4. Segmentation of Gel Image

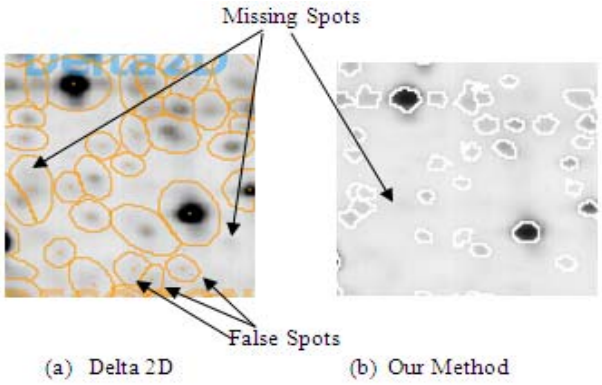


Figure 3. A part of gel image showing some missing spots and false spots using Delta 2D and using our method

VI. CONCLUSION AND FUTURE WORK

A novel method to detect protein spots in 2DE gel images has been presented. The over-segmentation problem of watershed transform has been addressed with spot characterization in the wavelet domain as well as in the watershed domain itself. As an advantage over commercialized software, our method uses only single threshold parameter. Detection of faint spots without introducing artifacts is still a problem for our method as well as commercialized software. Our future work will concentrate on it.

REFERENCES

[1] L.Anderson and N.Anderson, "Some perspectives on two-dimensional protein mapping," Clinical Chemistry, vol.30, pp.1898-1905, Dec.1984.

[2] P.H. O'Farrell, "High resolution two-dimensional gel electrophoresis of proteins," J.Biological Chem., vol.250, pp.4007-4021,1975.

[3] K.Kaczmarek et al, “Preprocessing of two-dimensional gel electrophoresis images,” Proteomics, vol.4, pp.2377-2389, 2004.

[4] Pierre Marie Nugues, “Two-Dimensional Electrophoresis Image Interpretation,” IEEE Trans. On Biomedical Engineering, vol.40, no.8, pp.760-770, August 1993.

[5] P. Cutler, G. Heald, I. R. White, and J. Ruan, “A novel approach to spot detection for two-dimensional gel electrophoresis images using pixel value collection,” Proteomics, vol. 3, pp. 392–401, 2003.

[6] Yi-Sheng Liu et al, “Spot detection for a 2-DE gel image using a slice tree with confidence evaluation,” Mathematical and Computer Modelling, vol.50, pp.1-14, 2009.

[7] Ramakrishnan Kazhiyur-Mannar, Dominic J. Smiraglia, Christoph Plass, Rephael Wenger, “Contour Area Filtering of two-dimensional electrophoresis images,” Medical Image Analysis, vol. 10, pp. 353-365, 2006.

[8] Laurent Najman, Michel Couprie, Gilles Bertrand, “Watersheds, mosaics, and the emergence paradigm,” Discrete Applied Mathematics, vol. 147, pp. 301-324, 2005.

[9] L. Vincent and P. Soille, “Watersheds in digital spaces: An efficient algorithm based on immersion simulations,” IEEE Trans. Pattern Anal.Mach. Intell., vol. 13, no. 6, pp. 583–598, Jun 1991.

[10] F. Meyer and S. Beucher, “Morphological segmentation,” Journal of Visual Communication and Image Representation, vol. 1, no. 1, pp. 21–46, Sept. 1990.

[11] K. Haris, S. N. Efstratiadis, N. Maglaveras, and A. K. Katsaggelos, “Hybrid image segmentation using watersheds and fast region merging,” IEEE Trans. Image Processing, vol. 2, pp. 1684–1699, Dec. 1998.

[12] P. Salembier and M. Pardas, “Hierarchical morphological segmentation for image sequence coding,” IEEE Trans. Image Processing, vol. 3, pp. 639–651, Sept. 1994.

[13] S. Mallat, “A Theory for Multiresolution Signal Decomposition: The Wavelet Representation,” IEEE Transactions on Pattern Analysis and Machine Intelligence, vol.11, pp. 674–693, July 1989.

[14] Kovacevic, J., Vetterli, M., “Non-separable multidimensional perfect reconstruction fillter banks and wavelet bases for Rn,” IEEE Trans. Inform. Theory, vol. 38, pp. 533-555, 1992.

[15] Barlaud, M., Sole, P., Gaidon, T., Antonini, M., Mathieu, P., “Pyramidal lattice vector quantization for multiscale image coding,” IEEE Trans. Image Process., vol. 3 (4), pp. 367-380, 1994.

[16] W.Sweldens, “The lifting scheme: A custom-design construction of bioorthogonal wavelets,” Applied and Computational Harmonic Analysis, vol. 3, pp.186-200, 1996.

[17] Jelena Kovacevic and Wim Sweldens, “Wavelet Families of Increasing Order in Arbitrary Dimensions,” IEEE Transactions on Image Processing, vol. 9(3), pp. 480-496, March 2000.

[18] Ratnesh Singh Sengar, A. K. Upadhyay, Pushkar G. Patwardhan, Manjit Singh, Vikram M. Gadre, “Approaches based on non-separable filter banks in 2D Gel electrophoresis image analysis,” Proceedings of Asia Pacific Signal and Information Processing Association (APSIPA) International Conference, p.p. no. 387-392, December 2010.