Dynamic analysis and recognition of cell-cycle screening based on morphological structures

Dynamic Analysis and Recognition of Cell-Cycle Screening Based on Morphological Structures

Donggang Yu[1,2] , Tuan D. Pham[1,2] Hong Yan[3] and Denis I Crane[4]

[1]School of Information Technology,
[2] Bionformatics Applications Research Centre
James Cook University Townsville, QLD 4811, Australia

[3]Department of Electronic Engineering,
City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong

[4]School of Biomolecular and Biomedical Science,
Griffith University, Nathan, Brisbane, Qld 4111, Australia

Abstract

In this paper, some new and efficient algorithms are described for dynamic analysis and recognition of cell-cycle screening based on morphological structures. The series of morphological structural points are described. The morphological structural models are approached, and they can be used for shape analysis and recognition of different cell phases.

Key Words: Cell-screening, morphological structure, recognition of image contour shape, analysis of morphological model.

1. Introduction

In order to assist scientists to understand the complex process of cell division or mitosis [1]-[5], analysis of cell-cycle screening by automated fluorescence microscopy is used. An essential task for cell-cycle screening is to measure cell cycle progression (inter phase, prophase, metaphase, and telophase) which can be identified by measuring nuclear changes. The most difficult task of such analysis [6]-[8] is finding different stages which can be presented by nuclear size and shape changes during cell mitosis. For example, the nuclear migration during cell division is shown in Fig. 1. An adaptive logical method for binarization [13] is used to separate binary cell images in cell-cycle screening. They are shown in Fig. 2. The description of image contour plays an important role for the shape analysis and recognition of image. line segment, critical points and their convexity and concavity are useful features to analyze the shape of image contour. Many methods and algorithms are developed for the description of contours in the past [9]-[12]. However, these descriptions cannot form series of sets, or the inter contour of a binary image can not be processed based on these algorithms [11], which make the analysis and understanding of contour shape difficult. Also, no one uses difference code to describe and extract these series of features. The methods proposed in this paper can make it possible. Some useful morphological structures of cell images are are described in Section 2. Analyzing and recognizing shape and size of cells in different phases are discussed in Section 3. Finally, a conclusion is given.

2. The morphological structure of different cell phases

2.1. Preprocessing of cell images

The binary contours of cell images of different phases (see the bottom-left cells in Figs.2(1-8)) can be followed based on binarization cell images, and shown in Figs. 3(1-8) respectively. Let the starting point of an binary image be the upper-left corner. Freeman code is used, and the contours are 8-connected. The direction of contour following is counter clockwise.

The chain code set of contour k is represented as:

$$C_k = \{c_0, c_1 ... c_i, ... c_{n-1}, c_n\} \quad (1)$$

Proceedings of the Sixth International Conference on Intelligent Systems Design and Applications (ISDA'06)
0-7695-2528-8/06 $20.00 © 2006 IEEE

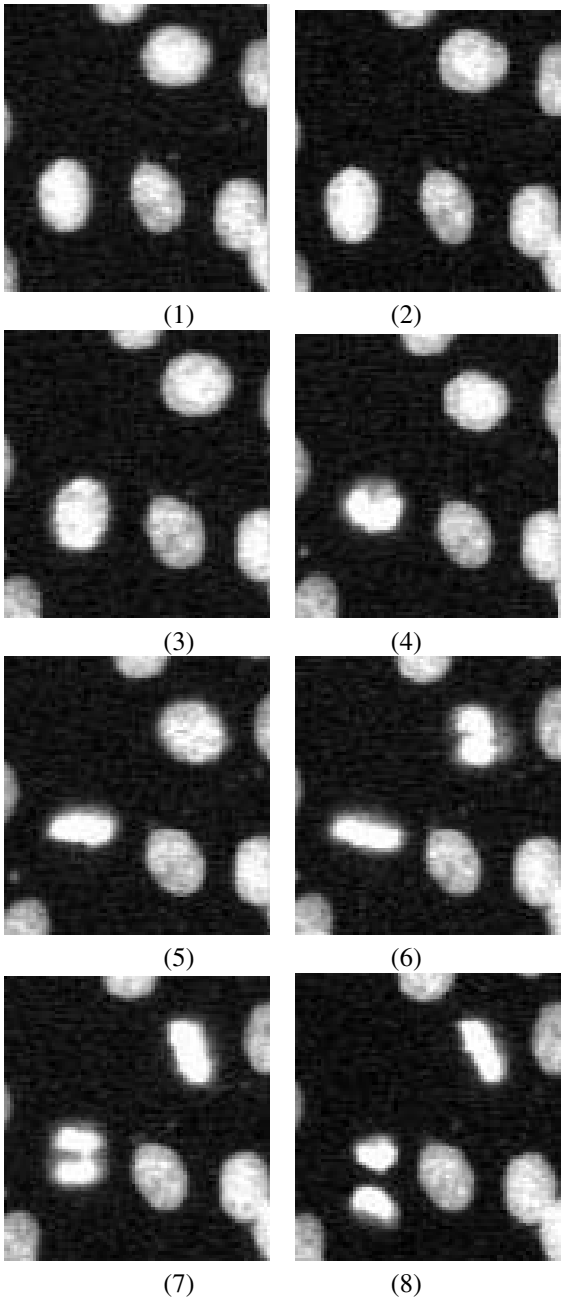

Figure 1. One example of cell-cycle screening.

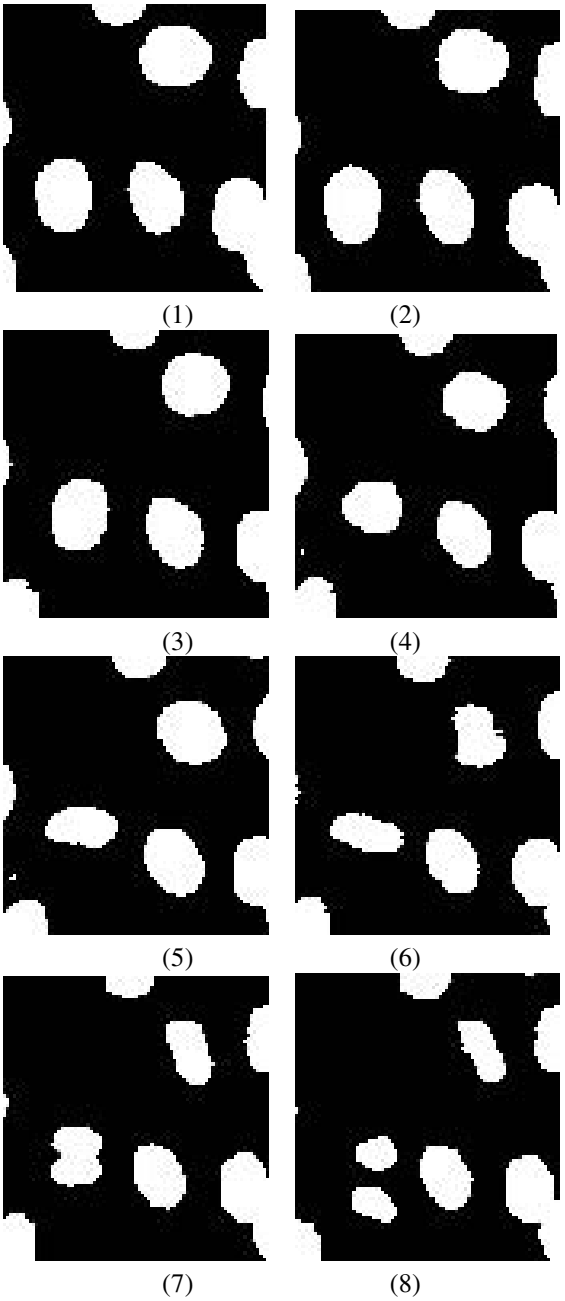

Figure 2. Binarization of cell images in one cell-cycle screening.

Proceedings of the Sixth International Conference on Intelligent Systems Design and Applications (ISDA'06)
0-7695-2528-8/06 $20.00 © 2006 IEEE

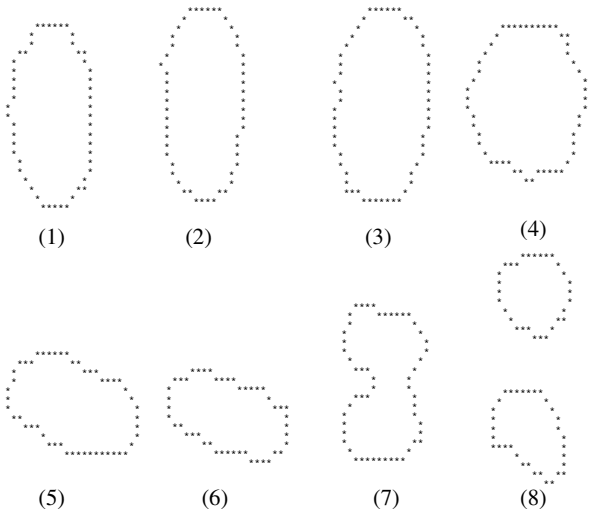

Figure 3. Cell contours of different phases (bottom-left cell in the cell-screen).

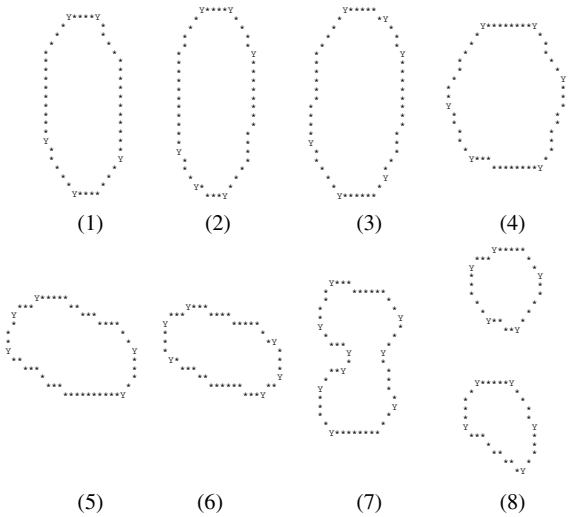

Figure 4. Smooth following and linearization of contours in Fig. 3.

where i is the index of the contour pixels. The difference code, d_i, is defined as:

$$d_i = c_{i+1} - c_i. \quad (2)$$

In smooth followed contours, $|d_i|$ equals 0 or 1 [12]. The smoothed contour can be converted to a set of lines which consist of ordered pixels. Suppose that the direction chain code set of the smoothed contour is

$$\{c_l^{ln}[i] \quad (i = 0, ...(n_l^{ln} - 1))\}, \quad (3)$$

where ln is the ln-th line of a smoothed contour and n_l^{ln} is the number of points of the ln-th line. A linearized line has the following property: [12]
if

$$d_{ij} = c_l^{ln}[i] - c_l^{ln}[j] \quad (i = 0, ...k-1), (j = 0, ...k-1), \quad (4)$$

then

$$\mid d_{ij} \mid \leq 1 \quad (i = 0, ...k-1), (j = 0, ...k-1). \quad (5)$$

Therefore, a linearized line contains only two elements whose chain codes meet Equation (5). Two element codes of the linearized line are represented by $cdir1$ and $cdir2$ respectively[12]. The contours in Figs. 3(1-8) are followed smoothly and linearized. They are shown in Fig. 4 where the spurious points in contours are removed and character "Y" is the first point of each linearized line.

2.2. Structural points of smoothed contours

The structural points are some special points which can be used to represent convex or concave change in the direction of chain codes between two neighboring lines along the contour. Their definition and detection are based on the structure patterns of element codes of two lines. Assume that $line[ln]$ is the current line and that $line[ln-1]$ is the previous line.

Definition 1. The convex point in the direction of code 4 (represented with the character "∧")
If the element codes 3, 4 and 5 occur successively as a group of neighborhood linearized lines, then one convex point can be found as follows:
if $cdir1$ of $line[ln]$ is code 4, $cdir2$ is code 5 and the direction chain code of the last pixel of $line[ln-1]$ is code 3, then the first pixel of the current line $line[ln]$ is a convex point which is represented with "∧".

Definition 2. The concave point in the direction of code 4 (represented with the character "m")
If the element codes 5, 4 and 3 occur successively as a group of neighborhood linearized lines, then one concave point can be found as follows:
if $cdir1$ of $line[ln]$ is code 4, $cdir2$ is code 3 and the direction chain code of the last pixel of $line[ln-1]$ is code 5, then the first pixel of the current line, $line[ln]$, is a concave point which is represented with "m". Similar to Definitions 1-2, other structural points can be defined and found. These points are convex points "v", "[', ")", "F", "o", "T", "s", and concave points "$", "]", "(", "f", "O", "t" and "S" which are shown in Fig. 5 respectively. These structural points describe the convex or concave change in different chain code directions along the contour, and they can therefore be used to represent the morphological structure of contour regions. We can see that though the

Proceedings of the Sixth International Conference on Intelligent Systems Design and Applications (ISDA'06)
0-7695-2528-8/06 $20.00 © 2006 IEEE

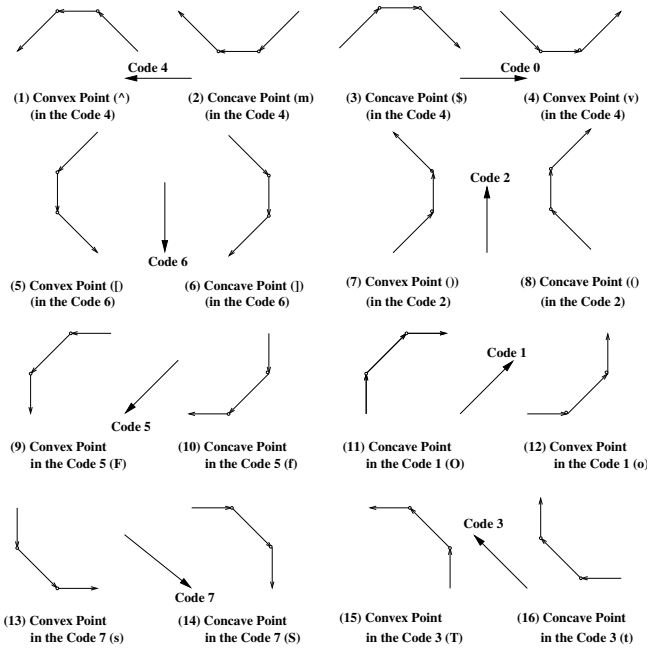

Figure 5. Structural patterns of structural points.

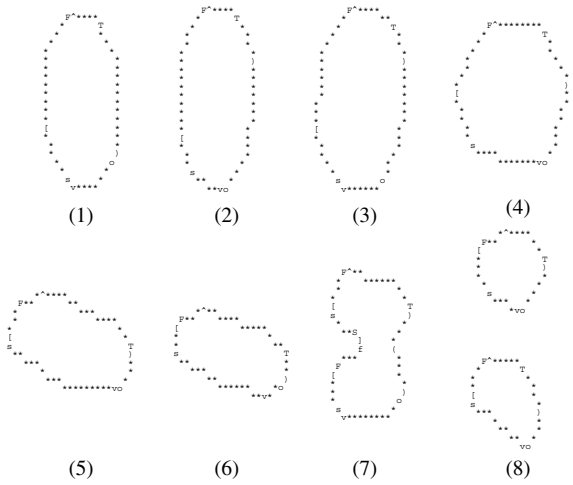

Figure 6. Cell morphological structures of different phases (bottom-left cell in the cell-screen.

representing shape of structural point "∧" is same that of structural point "v", but they are different. The structural point "∧" describes convex shape, but "v" concave. Such a property is useful for shape description.

The series of structural points of cell images (see Fig. 4) can be found and shown in Fig. 6 based on the above algorithm (see the patterns in Fig. 5). For the outer contour in Fig. 6(1), the series of structural points is:

"∧" → "F" → "["→ "s" → "v"→ "o"→ ")" → "T". It is clear that the contour shape is convex polygon, where it can be approximately defined as a ellipse.

For the outer contour in Fig. 6(7), the series of structural points is:

"∧" → "F" → "[" → "s"(convex) → "S" → "]" → "f" (concave) → "F"→ "["→ "s" → "v"→ "o"→ ")" (convex) → "(" (concave) → ")"→ "T" (convex).

It is clear, that the above contour shape consist of of two pairs of convex and concave change.

3. Dynamic analysis and recognition of different cell phases

In order to detect the morphological structures of cells, it is necessary to recognize the cell shape of different cell phases. We can see that there mainly two classes of cell shapes, ellipse and barbell, for our cell screening. The ellipse shapes can be three types, skew, horizontal and vertical based on relation between their major and minor axes. It is clear that here "skew", "horizontal" and "vertical" are fuzzy. Some recognition models of cell shapes can be described as following based on their morphological structures:

If a cell shape is a ellipse, there are no concave structural points on the outer contour of the cell contour. Furthermore, four models of ellipse can be described as following. Calculate number of group of codes, (codes 0, 4, 5 and 1), (codes 5, 1, 2 and 6), (codes 6, 2, 7 and 3), (codes 7, 3, 0 and 4), on the outer contour of the cell image contour respectively.

Morphological model 1: Ellipse shapes $e_{(5,1,2,6)}$ and $e_{(6,2,1,5)}$.

For these shapes, the number of group of codes, (codes 5, 1, 2 and 6), is largest.

Let $c_{5,6,1,2}$ be the total number of codes 5, 6, 1 and 2, c_t be the total number of all codes, $c_{5,1}$ be the total number of codes 5 and 1, and $c_{6,2}$ be the total number of codes 6 and 2, on the outer contour of the cell image respectively. If (1) the above Condition is met; (2) its outer contour mainly consists of chain codes 5, 6, 1 and 2 ($c_{5,6,1,2} \geq \frac{1}{2}c_t$); (3) the number of chain codes 5 and 1 is more than that of chain codes 6 and 2 ($c_{5,1} \geq c_{6,2}$), then the cell image shape is recognized as the shape $e_{(5,1,2,6)}$, otherwise ($c_{5,1} < c_{6,2}$) the cell image is recognized as the shape $e_{(6,2,1,5)}$.

In this model, the cell image shape, $e_{(5,1,2,6)}$, is a skew ellipse in the direction of code 5 or 1, and the cell image shape, $e_{(6,2,1,5)}$, is a vertical ellipse mainly.

Morphological model 2: Ellipse shapes $e_{(7,3,0,4)}$ and

Proceedings of the Sixth International Conference on Intelligent Systems Design and Applications (ISDA'06)
0-7695-2528-8/06 $20.00 © 2006 IEEE

$e_{(0,4,3,7)}$.
For these shapes, the number of group of codes, (codes 7, 3, 0 and 4), is largest.
Let $c_{7,0,3,4}$ be the total number of codes 7, 0, 3 and 4, c_t be the total number of all codes, $c_{0,4}$ be the total number of codes 0 and 4, and $c_{7,3}$ be the total number of codes 7 and 3, on the outer contour of the cell image respectively. If (1) the above Condition is met; (2) its outer contour mainly consists of chain codes 7, 0, 3 and 4 ($c_{7,0,3,4} \geq \frac{1}{2}c_t$); (3) the number of chain codes 7 and 3 is more than that of chain codes 0 and 4 ($c_{7,3} \geq c_{0,4}$), then the cell image is recognized as the ellipse shape $e_{(7,3,0,4)}$, otherwise ($c_{7,3} < c_{0,4}$) the cell image is recognized as the shape $e_{(0,4,3,7)}$.
In this model, the cell image shape, $e_{(7,3,0,4)}$, is considered as a skew ellipse in the direction of code 7 or 3, and the cell image shape, $e_{(0,4,7,3)}$, a horizontal ellipse.
Morphological model 3: Ellipse shapes $e_{(6,2,7,3)}$ and $e_{(7,3,2,6)}$.
For these shapes, the number of group of codes, (codes 6, 2, 7 and 3), is largest.
Let $c_{6,7,2,3}$ be the total number of codes 6, 7, 2 and 3, c_t be the total number of all codes, $c_{6,2}$ be the total number of codes 6 and 2, and $c_{7,3}$ be the total number of codes 7 and 3, on the outer contour of the cell image respectively. If (1) the above condition is met; (2) its outer contour mainly consists of chain codes 6, 7, 2 and 3 ($c_{6,7,2,3} \geq \frac{1}{2}c_t$); (3) the number of chain codes 6 and 2 is more than that of chain codes 7 and 3 ($c_{6,2} \geq c_{7,3}$), then the adjunctive segment is recognized as the ellipse shape $e_{(6,2,7,3)}$, otherwise ($c_{6,2} < c_{7,3}$) the cell image is recognized as the shape $e_{(7,3,2,6)}$.
Morphological model 4: Ellipse shapes $e_{(5,1,0,4)}$ and $e_{(0,4,5,1)}$.
For these shapes, the number of group of codes, (codes 5, 1, 0 and 4), is largest.
Let $c_{4,5,0,1}$ be the total number of codes 4, 5, 0 and 1, c_t be the total number of all codes, $c_{5,1}$ be the total number of codes 5 and 1, and $c_{4,0}$ be the total number of codes 4 and 0, on the outer contour of the cell image contour respectively. If (1) the above conditions are met; (2) its outer contour mainly consists of chain codes 4, 5, 0 and 1 ($c_{4,5,0,1} \geq \frac{1}{2}c_t$); (3) the number of chain codes 5 and 1 is more than that of chain codes 4 and 0 ($c_{5,1} \geq c_{4,0}$), then the cell image shape is recognized as ellipse shape $e_{(5,1,0,4)}$, otherwise ($c_{5,1} < c_{4,0}$) the cell image shape is recognized as the shape $e_{(0,4,5,1)}$.
Based on the above recognition models, the cell image in Fig. 6(1) is recognized as shape $e_{(6,2,7,3)}$, the cell images in Figs. 6(2-3) are recognized as shape $e_{(6,2,5,1)}$ and the cell images in Figs. 6(5-6) is recognized as ellipse shape $e_{(0,4,3,7)}$.
Morphological model 5: Barbell shapes.
If (1) there are two concave structural changes; (2) there is one pair of corresponding concave structural points, "∧" and "$" (horizontal), "]" and "(" (vertical), "f" and "O" (skew), or "t" and "S" (skew), then cell image contour can be recognized as the barbell shape. There are four types of barbell shapes which can be defined based on which pair of concave structural points is found. The cell image contour in Fig. 6(7) can be recognized as a barbell shape, and its pair of corresponding concave structural points are structural points "]" and "(".
Also, other morphological structures of cell images can be described.
For these morphological structures of cell images, their structure size information is considered, and each type of shape can be fined. For example, different ellipses can be recognized based on the rate between major and minor axes, and number of contour points of cell image.
Similarly, other morphological structures of cell images in cell-screen can be constructed. Based on the above models, morphological structures of different cell phases can be analyzed as follows:
(1) if (a)the cell shapes are a ellipse shape; (b) the number of points in cell outer contour is large enough (threshold can be found from statistical analysis), then the cell is a normal changing one and its changing trend is that the rate between major and minor axes of the cell image is decreased with time (metaphase).
(2) if the rate between major and minor axes of the cell image is large enough (threshold can be found from statistical analysis), then the cell will be split soon (telophase).
(3) if the cell shapes are a barbell shape, then then the cell will be split (telophase).
(4) if (a) the cell shapes are a ellipse shape; (b) the rate between major and minor axes of the cell image is about 1:1; (c) the number of points in cell outer contour is little enough (threshold can be found from statistical analysis), then the cell is a newly changing one, and its changing trend is that the number of points in cell outer contour is increased with time (prophase).
Based on the above analysis, the cells in Figs. 6(1-5) are at metaphase, the cells in Figs. 6(6-7) are at telophase soon, and the cells in Fig. 6(8) are at prophase.
In this way, cell-screening can be analyzed and recognized dynamically and automatically.

4. Conclusion

An efficient and new method has been developed for dynamic analysis and recognition of cell-cycle screening based on morphological structures. The algorithm of extracting structural features (structural points) is described based smooth followed contour, linearized line and difference chain codes. The morphological structures of cell contour shapes are constructed based on the structure points, difference chain code of linearized line and geometry fea-

tures. The dynamic analysis and recognition of cell-cycle screening are approached based on morphological structure models and prior knowledge of cell images. The Compare our algorithm with other methods [1-12], the best useful contribution is that some series of structural features of linearized lines of contours are found and morphological models of cell contour shapes are described based on our algorithm, but other methods not. Our method is efficient and new because morphological structure models of cell images are constructed, and these models simulate artificial intelligence.

Acknowledgement- This work is supported by the Australia Research Council ARC-DP grant (DP0665598).

References

[1] Fox, S.: Accommodating cells in HTS. Drug Discovery World, **5**, pp. 21–30, 2003.

[2] Feng, Y.: Practicing cell morphology based screen. European Pharmaceutical Review, **7**, pp. 7–11, 2002.

[3] Dunkle, R.: Role of image informatics in accelerating drug discovery and development. Drug Discovery World, **5**, pp. 75–82, 2003.

[4] Yarrow, J.C., et al.: Phenotypic screening of small molecule libraries by high throughput cell imaging. Comb Chem High Throughput Screen, **6**, pp. 279–286, 2003.

[5] Hiraoka, Y., and Haraguchi, T.: Fluoresence imaging of mammalian living cells. Chromosome Res, **4**, pp. 173–176, 1996.

[6] Chen, X., Zhou, X., and Wong, S.T.C.: Automated segmentation, classification, and tracking cancer cell nuclei in time-lapse microscopy. IEEE Trans. on Biomedical Engineering, in press.

[7] Pham, T.D., Tran, D., Zhou, X., Wong, S.T.C.: An automated procedure for cellphase imaging identification. Proc. AI-2005 Workshop on Learning Algorithms for Pattern Recognition, pp. 52–29, 2005.

[8] Pham, T.D., Tran, D.T., Zhou, X., and Wong, S.T.C.: Classification of cell phases in time-lapse images by vector quantization and Markov models. Neural Stem Cel l Research, ed. Erik V. Greer, Nova Science, New York, 2006.

[9] Moktarian, F., Mackworth, A. K. A Theory of Multiscale Curvature-Based Shape Representation for Planer Curvature Angles. IEEE Trans. Pattern Analysis Mach. Intell. **14** (8), pp. 789–805, 1992.

[10] Fu, A. M. N., Yan, H., Huang K. A Curvature Angle Bend Function Based Method to Characterize Contour Shapes. Patt. Recog. **30** (10), pp. 1661–1671, 1997.

[11] Sonka, M., Hlavac, V. and Boyle R., "Image Processing, Analysis and Machine Vision," Chapman & Hall Computing, Cambridge, 1993.

[12] Yu, D., Yan, H. An efficient algorithm for smoothing binary image contours. Pro. of ICPR'96. **2** , pp. 403–407, 1996.

[13] Yang, Y., Yan, H. An adaptive logical method for binarization of degraded document images. Pattern Recognition **33(5)**, pp. 787–807, 2000.