Dam-based rolling ball with fuzzy-rough constraints, a new background subtraction algorithm for image analysis in microscopy

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Dam-based Rolling Ball with Fuzzy-Rough Constraints, a New Background Subtraction Algorithm for Image Analysis in Microscopy

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***Abstract*—Light microscopy measurements provide information on the concentration of molecules in cells, tissues, as well as whole mount. Microscope images as we acquire them are not ideal; images specifically often suffer from an uneven background due to illumination flaws. Consequently, for proper images analysis, background correction methods are required. Currently, the "rolling ball" method is often used for illumination correction. This method, however, is far from correct. It is less effective and robust in terms of noise elimination and it generates artificial artefacts in the image. In this paper we investigate correction methods and we propose a new method that employs a combination of fuzzy and rough set theory. The method constructs and constraints an improved version for the "rolling ball". Our results show that we obtained a better solution for background correction for light microscopy images. In addition, the method works more efficient compared to the existing approach.**

***Keywords*—Background subtraction, microscopy image analysis, dam-based, rolling ball, fuzzy-rough set**

I. Introduction

The study of biological phenomena using fluorescent imaging has transformed fluorescence from a qualitative localization test to quantitative tools for functional analysis giving rise to, for instance, features such as distance, area, velocity and intensity. Here we study quantitative measurements derived from digital images produced by an imaging system. A digital image of a specimen is created by the detector in the optical system. Inevitably, errors occur in the process of image acquisition via the specimen, the microscope or the detector itself [1]. An important source of error is the inhomogeneity of intensity in the background. Sometimes the fluorescence has a shorter exposure time which will result in capturing less emitted photons compared to a procedure of longer exposure time [2]. Collection of fewer photons means that the relative contribution of the noise increases. Additionally, background intensity will also accumulate through the surrounding fluorescence sources. This results in an undesirable contribution of the background with respect to the signal of the interest so that the intensity values of the digital image are, de facto, the sum of signal plus the background intensity.

In order to apply quantitative measurements in microscopy, a notion of the background must be known and, if possible, it must be removed before doing measurements in the image. Therefore, it is always desirable to subtract the inhomogeneous background. Several techniques have become available to deal with abovementioned problems. The common approaches serve to reduce the amount of inhomogeneity in the digital images, are known as background subtraction processing.

Existing methods in microscopy for illumination-based inhomogeneity correction are classified into two categories: acquisition-based methods and retrospective methods. In the methods of acquiring prior-knowledge, which usually employs the background image, a comparison sample is often made by defocusing or removing the specimen from the field of view [3]. Yielding results by linear image calculator, however, these methods cannot cope with objective shading, e.g. shading caused by variation in specimen thickness at transmission imaging or by a non-planar surface in reflecting imaging [2]. The other class is referred to as the retrospective methods, and these methods mostly manipulate the data in both the time-domain and frequency-domain using different sorts of filters, e.g. low (high)-pass-filter, linear-filter, etc. The drawback of these methods, however, is the limitation of the objects size and the comparative background scale. The background is assumed to be either darker or brighter than the foreground. Moreover, the overlap of objects with the background is kind of forbidden in restoration, otherwise the mixture of foreground signal will be eliminated in while applying the corrections in the frequency domain. Meanwhile, a mathematical morphology structuring element based on the image landscape has been introduced [4] [5], i.e. the rolling ball algorithm (RBA). With a certain radius, a virtual ball rolls over the ground of the topographical pixels. Each pixel that contacts with the surface of the ball will be selected for further processing. These methods, however, have limitations in that they are imprecise in the estimation of a solution and portray uncertainty in the control of the path in the application of microscopy image sets.

In this paper we introduce a novel approach which is based on mathematical morphology to achieve a good result in order to solve abovementioned problem in a more general way. Our method is unsupervised and employs the concept of fuzziness, and approximation of an assumption from rough set theory is used to constrain the objective function. Henceforth a dam is constructed to avoid the path of the RBA path to introduce topological distortion, and eliminate background while producing an enhanced foreground. The experimental results illustrate the efficient for images from both bright field and fluorescence microscopy are in achieved.

The paper is organized as follows: in Section II the materials and methods used for our approach are presented. In addition the relevant concepts and knowledge are reviewed. In Section III, description of the objective function and dam-building methodology is presented. Section IV provides the experimental result, followed by a discussion and conclusions in Section V.

II. Materials and Methods

A. Data acquisition

For our experiments we have used images from our database. One set of images is typical bright-field depicting cartilage cell cultures; these images were acquired with ta standard Zeiss Bright Filed microscope. The other set is a typical multi-channel fluorescence set depicting cultured cardiomyocytes; this set is acquired with the BD-Pathway Imager[6].

B. Fuzzy set theory

The notion of fuzzy set was introduced in 1965[7]. A fuzzy set is a class of objects that contains consecutive grades of membership, which value ranged from zero to one. This index assigned a "vague" characteristic of the set lead by the concept of level of belonging. Particularly, a conventional set, referred to as crisp set will have either a value of zero or one; i.e. just Boolean value. We start from a mapping function which in the method proposed by us can be written as:

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$$\mu_A: U \rightarrow [0,1] \qquad (1)$$

here the A is denoted as the fuzzy set. The mapping function μ_A is the membership function of A while U denotes the universe.

C. Rough set theory

Rough set theory is an approach to assess the imprecision and uncertainty; it is proposed by Pawlak [8]. Objects in the universe characterized by the same information, or knowledge are indiscernible (similar) in the view of available information about them. The concept of the indiscernibility relation is the mathematical basis of rough set theory. Here we will illustrate some of the basic concepts that are directly relevant to our research.

An information system is a pair S=$\langle U,\ A,\ V,\ f\rangle$, where U is a non-empty finite set of N objects $\{x_1,\ x_2, \dots, x_N\}$ called the universe, and A is also a non-empty set of attributes, and V is a value set such that $a: U \rightarrow V_a$ for every $a \in V$. With every subset of attributes B of A, $B \subseteq A$, here defines an equivalence relation on U as:

$$I(B) = \{(x, y) \in U \times U: f_a(x) = f_a(y), \forall\ a \in B\} \quad (2)$$

Elements belonging to U that can satisfy this equation (relation) $I(B)$ are objects with the same value for attributes B and therefore these objects are indiscernible with respect to B. Moreover, an equivalence class containing the element x will be defined as $I(B)(x)$, in short $B(x)$. The classes of the equivalence sets are the basic concept of B. Given any subset of attributes B, any concept $X \in U$ can be approximately defined by employing two exact sets respectively referred to as the lower and the upper approximation sets:

$$B_*(X) = \{x \in U: B(x) \subseteq X\}$$
$$B^*(X) = \{x \in U: B(x) \cap X \neq \emptyset\} \qquad (3)$$

Assigning to every subset X of universe U, a subset $B_*(X)$ is referred to as the B-lower approximation of X, which can be classified as elements of X in the concept of B. While $B^*(X)$ is the upper approximation which most possibly belong to X given the knowledge B. The exactness based on the approximation set can be expressed by:

$$\alpha_B(X) = \frac{|B_*(X)|}{|B^*(X)|}, \text{for } X \neq \emptyset, \qquad (4)$$

this equation is referred to as the accuracy of the approximation, where |.| denotes the cardinality of the sets. The accuracy measure captures the degree of completeness of the knowledge about the set X. According to findings from the literature [9] we can obtain a measurement of the roughness index by rewriting the equation (4) as

$$\rho_r = 1 - \alpha_B(X) \qquad (5)$$

From this normalized definition it holds that for every B and $X \subseteq U$, if $\rho_r = 0$, then the boundary region set X is empty. From this moment on, it can be said X is B-definable, e.g. X is a crisp set with respect to the knowledge B. Otherwise, if $\rho_r > 0$, then this means that X is B-undefinable, e.g. X is rough or uncertain with respect to the knowledge B.

III. Dam-based rolling ball built up strategy

Here we connect the concept of rough set and fuzzy theory to the image domain.

A. The Rolling Ball Concept

The quantitative measurement of (pixel)-intensity is a mixture of signal and background noise. It can be well estimated by measuring the local background pixels in the region of interest[10]. This procedure can be depicted by:

$$F_{obj} = \sum_{m=1}^{m-N_{obj}} F_{obj} - N_{obj} \frac{\sum_{n=1}^{n=N_{bkg}} F_{bkg}}{N_{bkg}} \qquad (6)$$

Where F is the fluorescent signal measured at each pixel (*m, n)*, *obj* is the object, *bkg* is the selected background area or volume, and N is the number of pixels in the selected object or background. This equation describes the rolling ball algorithm in a mathematical way by computing the contribution of the background noise per pixel.

B. Depth of dam selection

Our method proposes an approach to constrain the path of the rolling ball in terms of a threshold value. A dam is built according to multi-thresholding values that are used to fill the convex valley. The processing can be divided into two steps, the first step is an extension of the ball radius in the direction of gravity, so that the ball can reach deep and narrow valleys and thereby remove noise. The next step is to construct a dam to prevent over-segmentation of the image by using a bimodal threshold method. Choosing a relatively small radius for the ball means that, the path of the ball will go as deep as the radius of the ball allows (cf. Fig. 2). This smaller radius will result in a loss of energy and detail in the original image. To that end, the proposed method will produce a much smoother image and eliminate the artifacts generated during the projection of the sphere (cf. eq. 6 and Fig. 3).

For the composition of the fuzzy set we need to start an initialization. Therefore we have to obtain the multi-threshold values in order to construct the dam in the image-landscape. Let x_{mn} be the pixel value with respect to an image with the size $m \times n$, and will obtain two average grey levels.

$$t_0 = \frac{\sum_m \sum_n x_{mn}}{i}, \quad x_{mn} < t$$
$$t_1 = \frac{\sum_m \sum_n x_{mn}}{j}, \quad x_{mn} \geq t \qquad (7)$$

where, i and j denote the number of occurrences of x_{mn} according to the threshold intensity-level t, and $i + j = m \times n$. Given by an initial threshold value t, the two average intensity-levels, t_0 and t_1, can be considered as local background. In this manner two sets X_{t_0}, X_{t_1} are obtained with element x, the relationship of the pixels $x \in X$, and its corresponding region should be directly depended on the change of the pixel values and the change in the local background. Therefore, the membership function, under the condition of these properties, means that the smaller the difference of the element pixel and its corresponding local background value, the larger the output of the membership will be. Notice that, it is expected that one element should either belongs to set X_{t_0} or X_{t_1}. Consequently, this will result in a membership output value in an interval of 0.5 to 1. It is clear that the membership value equals one only if the element belongs to a crisp set. With this definition, we obtain thus:

$$g(x) = \begin{cases} \frac{x_{mn} - t_0}{I_{max} - I_{min}}, & x_{mn} < t \\ \frac{x_{mn} - t_1}{I_{max} - I_{min}}, & x_{mn} \geq t \end{cases} \qquad (8)$$

$$\mu_X(x_{mn}) = \frac{1}{2}[(g(x) - 1)^2 + 1] \qquad (9)$$

where the I_{max} and I_{min} represent the maximum and minimum image intensity value.. For a given value t, the membership value $\mu_X(x_{mn})$ is in the interval [0.5, 1]. Moreover, in a 2D line plot the membership function has a convex shape; this results in a slight change of slope near 0.5 with much vagueness near the fuzzy centre (value 0.5), and a dramatic change of slope near 1 with fast rate of convergence near the crisp point (value 1).

By definition, the fuzzy set is known as an approach to access the quantitative analysis of membership between the elements and sets. In this manner, we can obtain a performance measure of the fuzzy set segmentation result, which provides a better image landscape than the Otsu method, which is known to provide good uniformity evaluations. This is because uniformity is a measurement of indicating a degree of variance in a segmented region and the mean values belonging to this region. However, a shape evaluation is summary of a generalized gradient value of every pixel by checking the relationship between the determined threshold value and the grey values of its neighbouring pixels. Consequently, the appropriate the threshold is chosen, the better the resulting image landscape representation will be accomplished.

So we assume that for our bimodal thresholding method the result of the first segmentation will separate the background and foreground, thereby containing the objects of interest. For the type of images we are using this refers to the cell cytoplasm and the nucleus. We need to segment the cytoplasm and the nucleus in order to express these in terms of vagueness and roughness. For our applications, the most likely shape we therefore will obtain is one in which all foreground pixels should contain cytoplasm and nucleus (cf. Fig. 1). Successful partition of the nucleus from the maximum shape is required to define the boundary between the cytoplasm and the nucleus, and therefore, an upper-approximation set can be made as the pixels belonging to cytoplasm, whereas the nucleus is the lower-approximation set. In order to transform the image data into rough set data we need the appropriate operations.

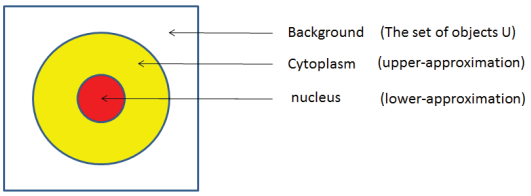


Fig. 1. Illustration of rough-set reflection in a cell image.

We now need to produce the upper-estimation of the rough set. We do this by assessing all neighbouring pixels for each pixel in the (original) image; defines as:

$$P_{upper}(x,y) = \frac{2}{\sqrt{n}}\left(\sum_{i=1}^{n}\left|P_{n-neighboring} - P(x,y)\right|^2\right)^{\frac{1}{2}} \quad (10),$$

where n is the number of n-neighbouring pixels for each pixel; in this paper we use n=8. The upper-approximation set is a collection of all points, which possibly belongs to one segmented region. In this manner a correlation of spatial information with respect to those who have same or similar values is built. The lower-approximation set contains original pixels that definitely belong to a class of known intensity, therefore can be considered as a set. Consequently, the roughness index ρ_r is formalized as:

$$\rho_r = 1 - \alpha_B(X) = 1 - \frac{P(x,y)}{P_{upper}(x,y)} \quad (11)$$

The value of the roughness index is large when the value of the upper-approximation is large compared with the original pixel value in the selected position. This typically occurs when there is large variance of the selected pixel with respect to its surrounding pixels. The intensity variation dramatically changes if there exists a boundary between two objects or regions. In other cases, the roughness index will be small, e.g. close to zero, as there is no significant change of intensity around a selected pixel.

In order to use the intersection of the properties of the fuzzy set and vagueness so as to constraint the membership function [11], we multiply eq. 9 and thus obtain:

$$g'(x) = \rho_r \,.\, g(x) \quad (12)$$

One should, however, pay attention to eq. 8, which consists of two partial equations that are depending on the local background, as well as a temporary threshold t, that needs to have a correlation index to allow continuous weighing. Therefore, we introduce a correlation function by taking the minimum and maximum intensity level into account, and weigh each component of membership function $g'(x)$ as:

$$\wedge = \frac{1}{2} \,.\, \frac{I_{max} - I_{min}}{I_{maxval}} \quad (13)$$

It is easily seen that $\wedge$ is in <0, 0.5]. Now, eq. 9 can be rewritten to:

$$\mu_X(x_{mn}) = \mu_X\left(g'^{(x)}\right) = \mu_X(\wedge \,.\, g_{x_{mn}<t}{}'(x) + (1-\wedge) \,.\, g_{x_{mn}\geq t}{}'(x)) \quad (14)$$

The appropriate measurement of uncertainty is the key to evaluate the degrees of vagueness if an element (pixel) belongs to a certain set (region) or not. Several approaches have been proposed, but here, we propose an evaluation based on Shannon's function to solve the uncertainty problems.

From the information entropy theory, the measured entropy of the vagueness can also be experienced within a slightly changed definition [12]:

$$E(X) = \frac{1}{MNln2}\sum_m \sum_n S(\mu_X(x_{mn})) \quad (15),$$

where M and N represent the size of the image. Note that the eq. 15, i.e. Shannon's function, is monotonically decreasing in the interval [0.5, 1], but monotonically increasing in the interval [0, 0.5]. In this manner it is possible to minimize the lowest energy while the definition zone is set to the interval [0.5, 1].

B. Construction of dam

Using global threshold values t_0 and t_1, i.e. indicating depth of the dam, we can constrain the path for the rolling ball (cf. Fig. 2).

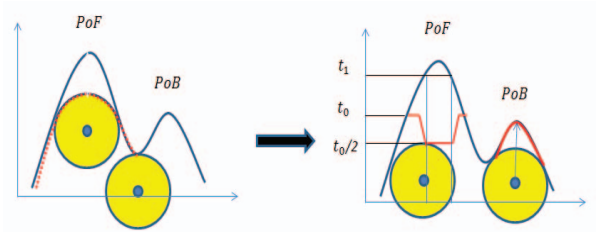


Fig. 2. Process of constructing dam in a 2D landscape. Ball path depicted as dotted line and full line respectively, in which the dotted line describes the way that original ball rolls under the image shape and full line shows the constrained path the ball can roll only according to the dam. Peak value of foreground and peak value of background are assumed as PoF and PoB respectively.

Now, t_0 and t_1 are the values for bimodal thresholding in terms of the foreground and the nucleus; in practice, the signal strength of the nucleus is often significantly higher compared to the cytoplasm and the background. The ball rolls under the valley and with the following rules: first, a judgment is needed if a hill (continues slope in the image landscape) belongs to the foreground (PoF) or background (PoB). The ball will not roll into the convex areas if the hill is recognized as PoF. If the value of the peak of the hill larger

than t_0, meaning suppression will occur if the ball is forced to rolling To that end, it is necessary to erect a dam to prevent the ball from over-correction. Subsequently, t_1 is set to smooth the slope of the dam in terms of enhancing the PoF region. The depth $(t_0/2)$ is chosen prior the processing (cf. Fig. 2)

The smoothing factor of the foreground during subtraction of the ball path is relative to the radius of the ball. Unlike the existing algorithm, which requires to be tuned for every step before adapting the ball to the object of interest, the proposed method is more robust as it includes an adaptive radius. In general, the radius should be chosen larger than the size of the smallest object after examination of the whole image for the biggest scale in the foreground.

IV. Experimental results

In order to illustrate the performance of the proposed method, a number of experiments are done on a database of both myocardial cell images (256 x 256 pixels, 16 bit) and images of cartilage cell cultures (1024 x 1024 pixels, 8 bit). The evaluation methods are implemented in qualitative and quantitative terms.

A. Qualitative tests

1) Artefact removal:

In Figure 3 myocardial muscle are cells depicted, and the images processed with an increasing ball radius. Information useful for the further processing is actually kept in the background and also well artefacts are enhanced. Artefacts will, in general, occur between the edge or the corner of an image due to the start centre of the rolling ball and results in an embedded effect of the rolling ball. This is shown in Figure 3 as irregular high energy (green and red).

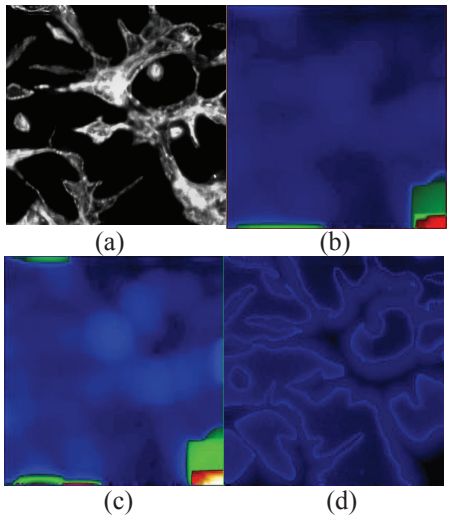


Fig. 3. (a) is part of a cardiomyocytes cell image; Heat map (b) and (c) is the background extracted by normal rolling ball method with radius 30 and 50; and (d) is the result of the new method.

From results shown above, the heat colours (non-blue) of the background image illustrate that the errors accumulate during the background correcting procedure. This significantly harms the further processing. In Figure 3 (b) and (c), we observe that there appears a square-like region of high errors when employing the existing rolling ball algorithm., Figure 3 (d), however, shows a much better result in terms of the elimination of the error.

The biggest drawback of the typical rolling ball method is that the choice of a radius, once set, might cause the artefacts if the ball cannot fit the size of objects in the image. From our results, it is clear that artefacts are perfectly eliminated, and the intensities across region (slope in the 2D line change) are enhanced for further analysis.

2) Multilevel background subtraction:

With the existing rolling ball method, there exists a strong correlation between the size of the radius of the ball and the result. It is, however, often very difficult to select a correct radius for a given image, or even for a larger set of images as is the case for the application of background correction in a high-throughput setting. By manually choosing and evaluating an objective size in every image (optimal parameters for the typical method) we can make a comparison with the proposed method that selects these parameters in an automated fashion. This is shown in Figure 4.

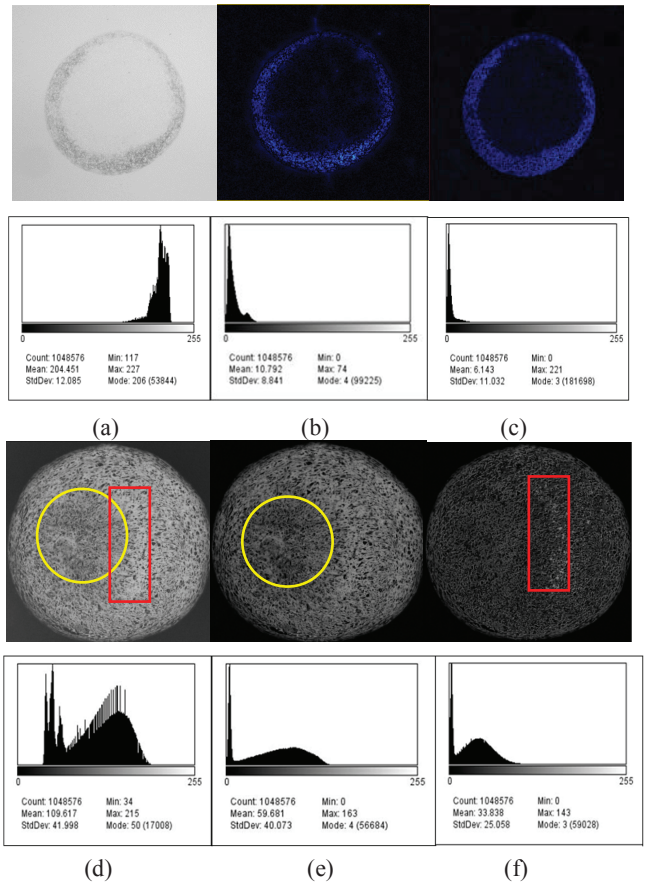


Fig. 4. In each column (a) to (c), and (d) to (e), which depict the original cartilage cell, existing background subtraction approach and proposed method, respectively. In the second row of each column is the global histogram of the corresponding data sets. Red rectangle shows the region of highlight remaining signal and the yellow circle depicts the over-extracting information.

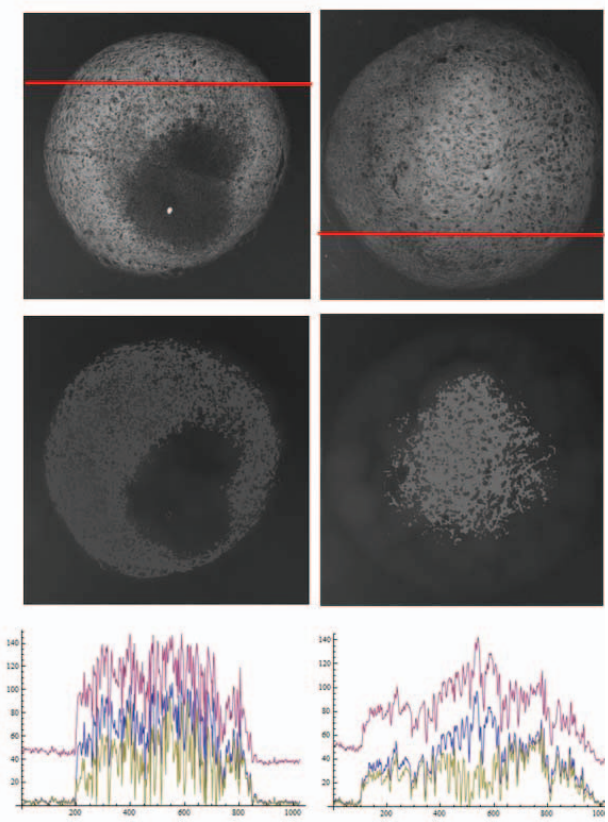
Figure 4 (a) and (d) show the cartilage cell images with severe bright illumination caused by wrong illumination setting. In Figure 5 (a) to (c), the testing method produced a result with a vague foreground that somehow lost clear visualisation of cartilage cells. However, our method generated a much clearer and smoother foreground shape that contains all relevant information. At the top of the ring of cells, a clearer and cleaner connection of cartilage can be appreciated through our method; the dam prevented the over elimination of mixed illumination. A more unambiguous and complete cartilage contour can be seen by visual inspection.

Note the specified regions in Figure 4 (d) to (f); the red region describes the ability of proposed method to remain all information in original foreground and the smoothed and evenly distributed background. On the contrary, in the yellow region, it is depicted

how the typical algorithm reduces lots of information, which is due to the ball path that rolls into a slope with lower curvature, causing reduction of useful information.

The partitioning of the differences in background illumination and foreground is quite difficult in most of the fluorescence and bright-field microscopy images. However, the proposed method performs very acceptable compared to the existing approaches; even with chosen optimal values - which is impractical under normal circumstances. In the proposed method the information is retained better while the distribution of the global intensity is more coherent. This can also be seen from the higher mean intensity in the resulting image.

2) Background suppression:



(a) (b)

Fig. 5. Two tests images of cultured cartilage cells with different shapes. From top to bottom for each column (a) and (b) is the original image, background produced by the method proposed and the line chart separately. The graphs show the intensity profiles along the horizontal direction (depicted as a red line in first row of images) as superimposed on the corresponding original (red), result from experimental method (blue) and result from proposed method (grey) images.

Different cartilage stem cell pellets are processed with the abovementioned approaches. As a pellet develops and grows older, the mean intensity on surface areas increases. From an analysis of merged-background intensity profile graphs, one can see that compared with original images, both methods do constrain the background. However, the proposed method reaches the lowest valley value for each hill in the image landscape, meaning an almost fully corrected foreground would be obtained if every illumination correction is subtracted while the foreground is smoothed. Our proposed method will produce a much clear foreground as the dam is like a wedge-shape results in an enhancement of the hill.

The typical rolling boll algorithm will generate an intersection with the original intensity profiles (cf. Fig. 5a), which to some extend is a consequence of using a large ball radius size. In the graphs of Figure 5, we notice that blue lines are almost the same as red line, there only is a change in amplitude. No smoothing or single pixel weighing in the experimental method, which will result in degradation of the image intensities.

B. Quantitative evaluation

A series of quantitative evaluation tests are performed on the two data sets, a control group and an experimental group of cartilage pellets in different growth stages. A specific evaluation standard referred to as coefficient of joint variation (CJV) [13] is used. The CJV is characterized by invariance to uniform transformation, i.e., multiplicative and additive illumination.

The performance of the proposed background subtraction method is quantitatively evaluated by analysing the variation of global intensity before and after using the subtraction.

The results, presented as the difference of standard deviations and the reduction of the CJV after different background subtraction strategies are shown in Table 1. The background signal that we try to estimate in the original is an additive signal. Using a rolling ball method (RBA) will reduce the average intensity of an image. Therefore we do not need to estimate the average intensity. The standard deviation, on the other hand, will play an important role in the evaluation since it is the numerator of the CJV. The quality of inhomogeneity correction is assessed by the reduction of the value of CJV, even if the golden standard of segmentation for a specific image is not available [13].

By comparing our method with the typical rolling ball method (cf. ImageJ), it is obvious that reductions are accomplished with an average standard deviation of 30.02% and CJV of 40.48%. At every iteration step of our method a constant reduction of standard deviation is realized meaning that there is no additional illumination introduced. Moreover, a large percentage of CJV reduction clearly shows that the background information in the original image can be significantly minimized through our approach.

V. Discussion and Conclusion

Background illumination correction based on the rolling ball is a mathematical morphology approach to find an estimation of illumination imperfections. For a "ball" rolling under the image landscape, it holds that those pixels the ball cannot touch will be kept in a smoothed foreground; e.g. the local minima and the peak. The smaller the radius of the ball, the deeper the topographical shape can be touched, and vice versa. With the existing rolling ball (RBA) method there are some problems that effectuate uncertainty and imprecision in the resulting image. Apparently, without constraints to the path of the ball, useful signal can be eliminated due to the over-subtraction of the foreground. In addition, the method can introduce artefacts as projections of the shape of the ball in the resulting image.

In the proposed method, a dam is erected to constrain a path by utilizing both the fuzzy and rough set framework. The membership function of fuzzy sets can handle overlapping partitions; whereas the lower and upper approximations of rough sets can characterize the vagueness and incompleteness in its class definition [14]. An effective way in constructing a dam based of classification of image elements, e.g. the nucleus and cytoplasm of a cell. This will result in smoothing of the foreground information and a global minimization of the standard deviation. The method includes a weighing factor for balancing of the mutual information shared by foreground and background.

Table 1. Quantitative results of both rolling algorithm and proposed method on two sets of microscope image data, each includes 13 pairs of images. The reduction of intensity variations and illuminations within selected object features are illustrated by the difference of standard deviation Δ Std and the coefficient of CJV.

T3-treated Group				Control group			
Image No.	Δ Std	CJV of Normal RBA	CJV of proposed method	Image No.	Δ Std	CJV of Normal RBA	CJV of proposed method
1	2.987	0.595	**0.515**	1	2.748	0.399	**0.320**
2	1.281	0.427	**0.393**	2	11.039	1.331	**0.890**
3	0.584	0.256	**0.240**	3	10.399	1.140	**0.735**
4	0.484	0.319	**0.305**	4	1.825	0.521	**0.500**
5	0.556	0.233	**0.218**	5	8.735	1.381	**0.956**
6	0.019	0.240	**0.238**	6	12.526	1.638	**0.927**
7	0.021	0.226	**0.224**	7	0.421	0.248	**0.230**
8	7.642	1.063	**0.752**	8	7.478	0.860	**0.640**
9	5.567	0.919	**0.746**	9	7.566	1.027	**0.800**
10	6.100	0.912	**0.710**	10	10.597	1.258	**0.819**
11	6.236	0.891	**0.730**	11	15.858	1.490	**0.877**
12	6.495	1.649	**1.419**	12	7.600	1.453	**1.190**
13	6.543	0.854	**0.695**	13	6.765	0.961	**0.787**

In this paper, we propose a dam-based rolling ball algorithm, which is a novel hybrid approach. The algorithm successfully overcomes the drawback of existing method and includes fully automated data driven parameter tuning. With the dam-constraint, the proposed method performs better in both qualitative and quantitative tests. We have applied the method to a set of myocardial muscle cell images and cartilage cell image. The results show that the proposed algorithm is robust to the noise, it does not introduce artefacts to the image and it is effective compared to the existing method. The new method is very promising for application to microscopy images in which further analysis is hampered by undesired background illumination effects.

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