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Quantifying the astrocytoma cell response to candidate pharmaceutical from F-ACTIN image analysis

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Abstract— **The distribution, directionality and motility of the actin fibers control cell shape, affect cell function and are different in cancer versus normal cells. Quantification of actin structural changes is important for further understanding differences between cell types and for elucidation of the effects and dynamics of drug interactions. We have developed an image analysis framework for quantifying F-actin organization patterns in confocal microscope images in response to different candidate pharmaceutical treatments. The main problem solved was to determine which quantitative features to compute from the images that both capture the visually-observed F-actin patterns and correlate with predicted biological outcomes. The resultant numerical features were effective to quantitatively profile the changes in the spatial distribution of F-actin and facilitate the comparison of different pharmaceuticals. The validation for the segmentation was done through visual inspection and correlation to expected biological outcomes. This is the first study quantifying different structural formations of the same protein in intact cells. Preliminary results show uniquely significant increases in cortical F-actin to stress fiber ratio for increasing doses of OSW-1 and Schweinfurthin A(SA) and a less marked increase for cephalostatin 1 derivative (ceph). This increase was not observed for the actin inhibitors: cytochalasin B (cytoB) and Y-27632 (Y). Ongoing studies are further validating the algorithms, elucidating the underlying molecular pathways and will utilize the algorithms for understanding the kinetics of the F-actin changes. Since many anti-cancer drugs target the cytoskeleton, we believe that the quantitative image analysis method reported here will have broad applications to understanding the mechanisms of action of candidate pharmaceuticals.**

I. INTRODUCTION

The widespread adoption of confocal microscopy along with the development of fluorescent proteins such as green fluorescent protein have opened up vast new challenges and opportunities in image analysis, such as uncovering the interaction mechanisms of cell signaling pathways and predicting the effects of new pharmaceuticals on cells. To extract useful information from cells for evidence of underlying protein chemistry, we need to systematically quantify the locations and organization of fluorescence intensity patterns in cell images in order to make cross image and cross experiment quantitative comparisons. F-actin controls the behavior of cells and understanding how F-actin spatially reorganizes under the treatments of different compounds contributes to profiling the effects of a specific reagent or pharmaceutical on cells. Previous studies[1] of studying F-actin patterns have mostly been based on visual inspection, sometimes only involving counting the intensity of the fluorescently-labeled actin within the whole cell area. As far as we are aware, there has been no systematic way to either automatically extract the visually-distinctive F-actin patterns from microscope images or give quantifiable and comparable measurements among a set of images under different treatments. In this paper we introduce an image analysis framework for quantifying the F-actin reorganization pattern from confocal images after the cells were treated under different conditions. Section 2 gives an overview of some related work and a generalization of the problems left unsolved in our previous work[2]; section 3 describes the methodology we used for image analysis and quantification; section 4 reports the experimental conditions for image acquisition, validation, software implementation and illustrates some sample results. The final section draws conclusions and discusses future work.

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II. METHODS

A. Image Acquisition

Images were from a mouse astrocytoma cell line. Cells were treated with two reagents known to inhibit actin: cytochalasin B (cytoB) and Y-27632 (Y), three candidate pharmaceuticals: OSW-1, Schweinfurthin A (SA) and a synthetic cephalostatin 1 derivative (ceph), and a vehicle control, DMSO. The inhibitors and the candidate pharmaceuticals have been observed visually to cause discreet changes in F-actin organization. Fixed cell cultures were stained with DAPI to label nuclei and facilitate single cell segmentation, and Phalloidin to label F-actin. Samples were observed under confocal microscopy and we randomly picked four regions for image acquisition under each condition. Since the z dimension of these images was quite small compared to high resolution in x and y, the maximum intensity projection was sufficient for analysis. Therefore the following analysis was based on 2D.

B. Analysis Framework

In this paper we deal with the problem of what features to extract for quantifying the spatial distribution of a single species of protein, in this case F-actin. The main motivation for this work is that in biological research, both quantitative

analysis and visualization of images are necessary for optimal understanding of the samples. Moreover training the classification model on visually perceived structures limits the potential of computational analysis to quantifying only the visual cues and masks important sub-visual information that potentially exists in the images. In outline, a new methodology is proposed for analyzing F-actin images consisting of 2 parts:

1) Segment each image into different feature regions in a top down fashion. The image was first segmented into 3 major parts by performing point-wise fuzzy c-means (FCM) clustering on extracted feature vectors. FCM has been successfully used for unsupervised segmentation of biomedical images. Previous work by Chuang et al [3] shows its robustness to noise. Then each segmented part was subdivided either by sub-cellular location or by texture features. We computed the area of different classes of segments per image and quantified their pairwise ratio to obtain scalar quantification results;
2) Quantify multi-scale texture features of the cytoplasm regions;

Figure1 shows the flow chart of our analysis framework.

III. IMAGE ANALYSIS

A. Tri-Band Segmentation

Classification by intensity differences between different locations is the most intuitive way to model the cellular appearance since the gray scale at each pixel is in proportion to the corresponding local concentration of fluorescence in cells. Based on the observation of hundreds of images and the input from biologists , it was justifiable to segment the F-actin images into 3 parts based on multi-scale intensities: the bright actin (BA) regions mainly at the edges of cells, the less intensely stained region corresponding to the cytoplasm, and the remaining dark background with noise An example showing the segmentation of an F-actin channel image using this criterion is shown in Fig.2

Classification was performed on per pixel basis. A feature vector was constructed by capturing the pixel intensity at different scales. Blurring was undertaken with a 5x5 Gaussian kernel and standard deviation of 1. We repeated it 3 times and acquired the intensity as one feature component after each filtering. The original pixel intensity was combined with these 3 component to build the final feature vector. More specifically, let $G^{(n)}_{w\times w}(I))$ denote the execution of the gaussian filtering n times in a w by w neighborhood. Then the feature vector we use for clustering is given by:

$$\overrightarrow{V} = [p(i,j), p^1(i,j), p^2(i,j), p^3(i,j)]^T \qquad (1)$$

where $p^k(i,j)$ denotes the pixel value of $G^{(k)}_{w\times w}(I))$ at location (i,j). The intensity at a pixel is a mixture of intensities from different segments. In order to obtain the probability that a pixel belongs to each major part we used FCM to cluster the feature vectors into different groups and labeled the corresponding major parts with the group label. The resultant component image of each segment was computed by multiplying the original intensity with the pixelwise probability map of respective segment. In the work of Chuang et al [3], the spatial information was included into the FCM process while in our method the spatial information was gathered by the multiscale feature vector independently from the clustering. The choice of length of the feature vector was problematic. More folds of gaussian filtering led to higher feature dimension and higher accuracy in segmentation. However we needed to balance accuracy with computation cost and 3 seemed to be the optimal choice between these two considerations.

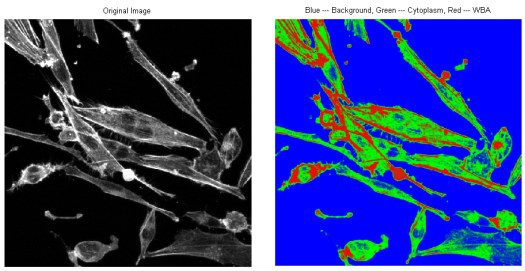

Fig. 2. This figure shows the original and segmented image of the cells under treatment of Y compound at a concentration of $5\mu M/ml$, the region labeled red are the bright actin(BA) regions, the region labeled green are the cytoplasm regions and the blue is the background

B. Stress Fiber Extraction

The less intense cytoplasm signal was further segmented into the stress fiber region and the remaining diffuse signal. Stress fibers(left sub-figure of Fig.3) are filaments embedded in the cytoplasm that appear more intense than the remaining signal, but less intense than the bright actin segment that generally corresponds cortical actin and punctuate actin.(right sub-figure of Fig.3) This suggests a strategy to extract stress

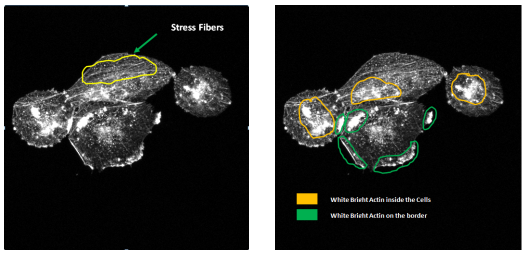

Fig. 3. The subfigure on the left illustrates the appearance of stress fibers in the cells and the subfigure on the right shows the appearance of cortical actin and punctuate actin

fibers by intensity thresholding only the less intensely stained region identified above by FCM. This was done by Otsu's method[4]. However the cytoplasm part could not be used directly since it was bordered by the remaining halos of the bright actin parts . Therefore the remaining halos were removed before detecting the stress fibers. This was achieved by computing the edges of the bright actin segments using the Sobel operator, then dilating these edges with a disk

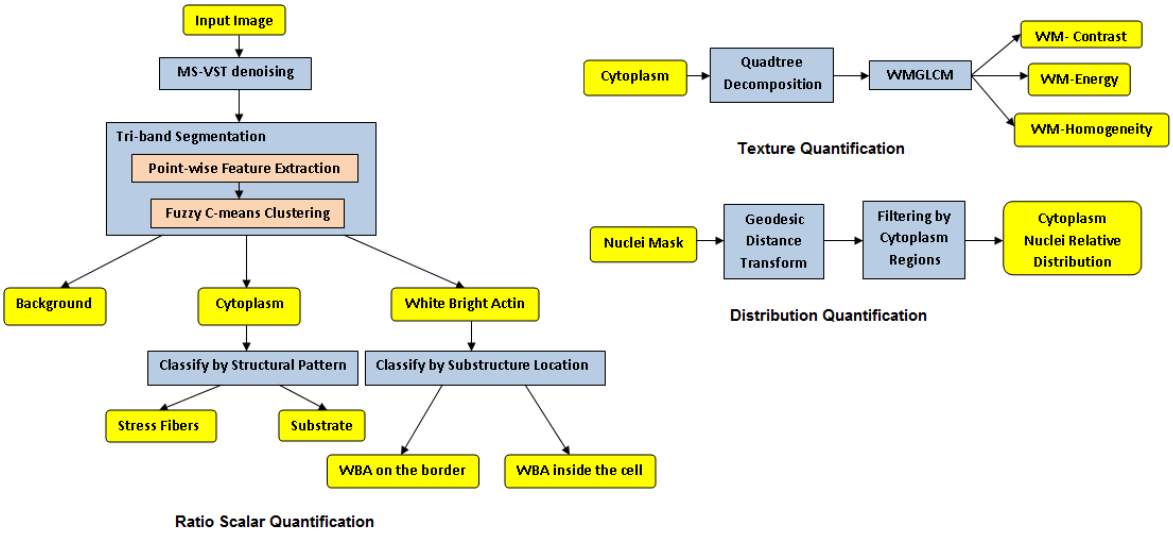

Fig. 1. The works shows the framework of our image analysis algorithms

shape structural element that completely covered all the halos remaining in the cytoplasm part and thus generating a mask for removing all the halos in the cytoplasm part. Due to the heavy blurring of the stress fibers due to the finite resolution of the confocal microscope, we also needed to enhance the halo free cytoplasmic parts of the images which are identified by I here:

i) $I_g = H_{log}(I)_{5\times5}$
ii) $I_r = I - I_g$
iii) $I_f = H_{unsharp}(I_r)_{\alpha=0.8}$

Here I denotes the halo free images, I_g denotes the image after LOG filtering. In the last step the resultant image from the subtraction goes through an unsharp filter[6] so that we get a mask for all stress fiber regions in the image.

C. Weighted Multi-scale Texture Analysis

The cytoplasm is a highly textured region. Therefore we applied a weighted multi-scale GLCM (Gray Scale Co-occurrence Matrix) (WMGCLM) to characterize the properties of the complex inner structure. The algorithm for WMGCLM is described as below:

Step 1 Apply quad-tree decomposition to the cytoplasm part

Step 2 For each scale, compute the GLCM for all the square segments belonging to that scale and get their contrast, energy and homogeneity, as shown in Eq.2

Step 3 Use the average of measurements from all the segments at one scale to form one measurement at that scale for the entire image.

Step 4 Weight the measurement from different scales by dividing by the elementary area at that scale and summing them up as shown in Eq.3

$$\begin{cases} GLCM_1 = \sum_{i,j} \mid i-j \mid^2 p(i,j), & \text{contrast;} \\ GLCM_2 = \sum_{i,j} p(i,j)^2, & \text{energy;} \\ GLCM_3 = \sum_{i,j} \frac{p(i,j)}{1+\mid i-j \mid}, & \text{homogeneity.} \end{cases} \quad (2)$$

$$WGLCM_k = \sum_{s=1}^{S} \frac{mean(GLCM_k^s)}{s^2} \quad (3)$$

where k denotes the index of the measurement; S denotes the maximum scale chosen, specifically 8 in our case.

Since we were mainly interested in SA, the following claim is used to check the quantification results. OSW1 and ceph have similar effects on F-actin as SA. We computed the following measurements for each image and put them into a feature vector:

1) The ratio of the border bright actin to the inner bright actin
2) The ratio of the border bright actin to the nuclei area
3) The contrast of the texture of the cytoplasm segment($WGLCM_1$)
4) The energy of the texture of the cytoplasm segment($WGLCM_2$)
5) The homogeneity of the texture of the cytoplasm segment($WGLCM_3$)

The quantification of each treatment was represented by the average of the 4 affiliated images. We held the 3 cases of SA treatment one by one each time and computed the Mahalanobis distance of its feature vector to the features vectors of all the remaining treatments. 3 distance bar graphs were obtained and thus we could know which treatment gave the closest response to SA under a certain concentration.

1) SA(10nM/ml) is the most similar to OSW1(1nM/ml)
2) SA(100nM/ml) is the most similar to ceph(1nM/ml)
3) SA(1000nM/ml) is the most similar to SA(100nM/ml)

IV. Results

We validated the quantification method by checking whether the quantification corresponds to expected biological outcomes and by visual verification. The subfigure on the left in Fig.4 shows an example for the quantification of the ratio of the stress fiber area to the cell area. The decrease of the amount of stress fibers could be validated visually from the

Fig.5. The two subfigures on the first line correspond to the DMSO, sa(10nM/ML) and the other two on the second line correspond to sa(100nM/ML) and sa(1000nM/ML).

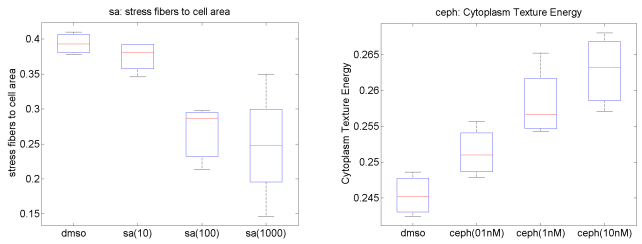

Fig. 4. The subfigure on the left shows the quantification result of the ratio of the stress fiber area to the cell area of SA; the one on the right shows an example of the box plot for the quantified texture energy of ceph.

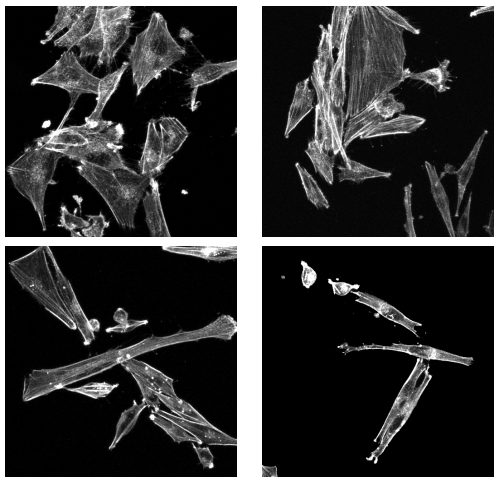

Fig. 5. This figures shows the images corresponding to the quantification result in the left sub-figure of Fig.4

The scalar ratio quantification and texture quantification are visualized via a box plot, displaying the mean and standard deviations of all the 4 images for each treatment condition. The 3 concentrations for each drug are listed from low to high left to right in one graph while the DMSO case was put on the left most as a reference. An example of these graphs is shown in the right subfigure of Fig.4

This figure highlights the differences in cytoplasm texture energy from one drug dosage to the next. This example can be generalized to other quantitative measurements of spatial distribution. Furthermore, this result additionally demonstrates that quantitative measurement can detect statistically significant differences that are not visually detected with any certainty.

The computed results well correspond to the known biological facts. This demonstrated that our quantification measurements can well profile to the F-actin pattern response to a certain medical treatment. Fig.6 visualizes the pair-wise mahalanobis distance between different treatment conditions(different pharmaceuticals or different concentrations) by a heat map colored matrix. The blue dots in each row

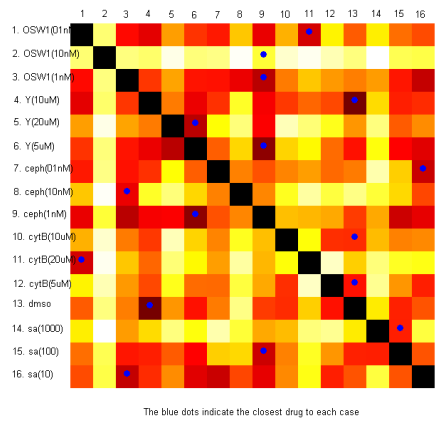

Fig. 6. This figure shows the pair-wise distance map of all the treatment conditions in our experiment. Darker color means smaller distance, in other word, greater similarity and black means the same.

corresponds to the closest case to the drug name on the left of the row.

V. CONCLUSION

In this paper we introduced an image analysis framework for quantifying the F-actin organization pattern in confocal microscopy images. The main problem we seek to solve is to determine which kind of features to extract and what measures should be taken for quantification so as to make the final results reliable, descriptive for the observation and discriminant for classification. Our method attempts to minimize the human bias of visual assessment definition as much as possible by choosing the intensity and locations as the main constituents of features. The final quantification result for all the images are grouped on individual pharmaceutical basis which made it easy to compare their effects. Future work will include running more validations to test the effectiveness of the quantitative measurements we choose and making modifications if necessary. Also we seek to find better quantitative descriptions of the features that is more catering to quantify F-actin pattern and adapt the system to analyze very large image databases.

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