Evaluation of Multiparameter Micrograph Analysis with Synthetical Benchmark Images

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Abstract—To analyze multiparametric images of biological systems in vivo, advanced image processing, visualization and datamining tools are under development. To evaluate the algorithms, fit the parameters and assess the algorithm’s accuracy, benchmark data sets supplied with a ground truth label are required. Because manual evaluation results, done by expensive experts are hard to achieve, new innovative strategies for evaluation are needed to allow the computer scientists to evaluate their algorithms. We propose an algorithm for the generation of synthetical multiparameter images of cell bodies. The algorithm generates a stack of intensity images, with different grey value characteristics from a reasonable set of parameters. Each image is composition of single synthesized grey value images. Tuning the parameters allow an individual incorporation of expert knowledge and supplies the developer with ground truth labels.

We describe and motivate the algorithm and present three applications of evaluation tools to synthetical micrographs. The results show the approach’s high relevance to application oriented image analysis in biomedicine.

Keywords—Biomedical Imaging, Multiparameter Image Analysis, Optical Microscopy, Object Detection, Image Analysis

I. INTRODUCTION

In recent years, much effort has been taken to analyze the functional relations of molecules in biological cells [1]. In this field, optical microscopy has drawn the increasing attention of the researchers for the last years considerably [2]. This is caused by (a) progresses in the identification of molecules by improved staining and imaging techniques and (b) the fact, that molecular interactions need to be observed in vivo to be understood. The latter demand is strongly motivated by the new established research field, called systems biology [3], where measurements of different cellular parameters, i.e. genome, transcriptome, proteome and metabolome, are integrated into cellular regulation models. In many studies the molecules must be allocated to cell compartments in the modeling step. Thus, techniques that are based on cell homogeny like micro-arrays and electrophoresis gels must be supplemented by methods of optical microscopy like it is done in many laboratories. This increased investment in digital imaging culminates in a demand for algorithms to support the analysis of these images.

In one class of imaging approaches, the analysis is a detection and/or classification of cellular intensity patterns, that are visualized using multistaining, multiband and multimodal (see [4]–[6] for example) imaging methods. These patterns indicate for instance local concentrations of molecules or structural membrane information.

In the development and evaluation of algorithms for image analysis, the evaluation and discussion of new approaches is often based on the application on benchmark datasets. A comprehensive collection of such sets can be found at the Computer Vision Homepage1. Most of these image data sets are recorded in an experimental set-up, showing faces, toys, fingerprints and traffic scenes.

A limited number of data sets show synthetical data for example to study texture, motion and stereo vision. In the field of biomedical image processing, benchmark data sets concentrate on macrobiological structures like mammograms2, gastrointestinal video endoscopy3 and MRI (Visible Human Project)4. The lack of images from the microbiological domain is caused by several reasons: First, the imaging apparatus are not integrated standardized products like in the macrobiological/medical domain. Thus, it may be awkward to find one data set, that is representative for all. Second, the biomedical researchers in this field do not share their micrographs easily with others because of competition and proprietary rights problems. Above all the last one, if the images content is settled in the field of drug discovery. Additionally, the dataset must contain a ground truth (or gold standard) i.e. a numerical expression of the correct evaluation result. For example, in case of a segmentation task, this would consist of a label image. In case of an object detection task, it would be a list of correct referential object positions. In the common case, such a ground truth is not available and must be simulated by a manual data evaluation by a human expert. But it is a well known fact, that in biomedical imaging, such human evaluations are expensive and error-prone [7]–[9]. The processing of synthetical micrographs is a promising loophole, because the usage of synthetical micrographs implies the supplements with a correct gold standard.

In this work, we propose a new benchmark data set of synthetical micrographs for the evaluation of micrograph processing

1http://www-2.cs.cmu.edu/ cil/v-images.html
2http://marathon.csee.usf.edu/Mammography/Database.html
3http://www.gastrointestinalatlas.com
algorithms. First, we discuss different approaches and describe our algorithm afterwards. Then, we present the application of image analysis algorithms to the data.

II. DIFFERENT APPROACHES

Starting point in generating synthetic image data is to find a good compromise between the simplicity of the object model and the similarity to real data sets. One approach is to simulate the biological elements, that are present in the experimental sample. Therefore, 3-dimensional geometrical models of the cells, the tissue, the present liquids, the hydrodynamic characteristics, the cell’s surface tension, the cell’s exchange of matter with their surround or the coupling of antibody markers to the cell have to be modeled. This model would have such an enormous complexity, that its implementation is beyond the scope of this work. Additionally the feasibility of such a reasonable modeling is to discuss on another level: How can such a system of tissue, cells, and cell functions be modeled, if some of them are subject to the top-level research field itself?

In fact, such an approach could be realized as an outcome of systems biology project itself.

Another approach would be to generate the micrograph data-driven. A set of real world image patches of cells is considered as a data distribution in a high-dimensional image space. A new, synthetic cell patch can be sampled from this distribution using for example Principal Component Analysis (PCA). This approach has the advantage that one would be enabled to simulate real world noise on the data. But this approach is not capable to create an entire micrograph of a set cells, that can overlap. There is no satisfying solution of the problem of composing single, quadratic image patches of single cells to one image.

In our approach, the micrographs are graphically generated, using a geometric model of a blood cell. One micrograph shows a set of $n_s$ fluorescent cells, given by their (i) positions, (ii) individual shapes and (iii) fluorescence distributions i.e. their grey values.

III. THE GRAPHICAL APPROACH

The a $n$-dimensional multiparameter image is generated as a stack of $n$ synthetic images of $m$ cells. All $n$ images have the same structure, i.e. cell bodies of identical shape and location, but different local intensity patterns reflecting different responses of imaging parameters. In each image one cell is drawn at $n_p, p = 1, \ldots, n$ positions $(x_o, y_o), (j = 1, \ldots, n_p)$. To determine the cell shape, a contour line of the cell is computed around the origins $(x_o, y_o)$ in a first step.

The contour line is computed around the cellular origin position $(x_o, y_o)$ using a radius function $r_{cell}(\varphi)$. The radius function determines the distance $r_{cell}(\varphi)$ from $(x_o, y_o)$ to the cell contour line for each given origin angle $\varphi$. The shape of the function is given by a sum of $n_G$ Gaussians $G_i(\varphi)$:

$$ r_{cell}(\varphi) = \sum_{i=0}^{n_G-1} G_i(\varphi), \text{ with}$$

$$ G_i(\varphi) = \rho_i \cdot \frac{1}{\sigma \sqrt{2\pi}} \exp{\left(\frac{\varphi - \mu_i}{2\sigma^2}\right)}.$$ 

The mean values are set equidistant to

$$ \mu_i = i \cdot \frac{2\pi}{n_G} \text{ for } 0 \leq i < (n_G - 1).$$

The curvature of the cell is controlled by the radius scaling factor $\rho_i$. These are set for each Gaussian individually to $\rho_i = \bar{r}_{cell} + \epsilon_{cell}(\mu_i)$, with $\bar{r}_{cell}$ as an average radius of the cell. The value $\epsilon_{cell}(\mu_i)$ is a uniform distributed random variable with zero mean and variance $\epsilon_{cell}$, that determines the individual shape of the cell (see Fig. 1 (a),(b)).

Two further contours are computed in an analogous way to the above. First, an inner contour function $r_{flu}(\varphi)$ is needed to simulate a non-uniform fluorescence distribution (dashed line in Fig. 1). Second, the border of the fluorescence corona of the cell is set by a third contour function $r_{cor}(\varphi)$ (dotted line in Fig. 1). For $r_{cor}$ the same function is used as $r_{cell}$, yet with an increased average radius. The $r_{flu}$ is computed with new initialized Gaussians, yet with a decreased radius.

After computing the contours, the intensities of the pixel grey values $g(x,y)$ inside the outermost contour $r_{cor}$ are computed. The can be considered as all pixels $(x,y)$ in the image, which are affected by the cellular parameters. First, for points of the three contours $r_{flu,cell,cor}$ and the origin $(x_o, y_o)$ the grey values are set to values $g_o + \epsilon, g_{flu} + \epsilon, g_{cell} + \epsilon$. 

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Fig. 1. To generate a synthetical micrograph, a set of cells are drawn, using a randomly warped geometric model. It is given through three contour lines (a), that are computed using shape function, which is a superposition of Gaussians (b). The grey values of the inner cell are set, using a polynomial defined grey value distribution from the center to the border (c), biased with random noise.
Fig. 2. The figure shows clippings from a foreground and background image and the result composite image. To compose a foreground image of a synthesized cell with a background image of pre-computed cells and/or random noise, the grey values of the pixels in both images are combined in a weighted sum to avoid non-realistic inhomogeneities. The weights \( w_c \) are computed dependent on the pixel coordinates, relative to the cell center.

Fig. 3. One example synthetical micrograph: The image consists of two layers. In a foreground layer fluorescent cells and non-fluorescent cells are combined. The background layer contains the same, but with reduced signal intensities, simulating out-of-focus structures.

and \( g_{\text{cor}} + \epsilon \). Again, \( \epsilon \) is random variable, simulating different fluorescence behaviors of different cells. Afterwards, the grey values for all remaining points of the cell are computed by interpolating the grey values of the contours. To compute the grey value for an inner cell point \((x, y)\) its radial distance \( r(x, y) \) and angle \( \varphi(x, y) \) is considered, illustrated in Fig. 1 (a). The grey value \( g(x, y) \) is computed as an interpolation of the grey values in the origin and in those three points on the three contour lines, that share the radial angle with the considered point \((x, y)\) (see black bullets in Fig. 1 (a)). For the interpolation a polynomial spline is used, as shown in Fig. 1 (c).

Finally, the generated cell body is set against the background in the micrograph. Fore- and background grey values are combined in a weighted sum to prevent strong, non-realistic step edges. To avoid a simple black background, a second image plane is computed with lower average grey values, which is taken as background and can be interpreted as out-of-focus blur. One subregion of one micrograph\(^5\) is shown far left in Fig. 4.

IV. SIMULATIONS

In this section we describe, how synthetical micrographs are used in three of our research subjects to illustrate their usefulness.

A. Cell detection in micrographs

In one project we develop a system for the automatic detection of immunofluorescent lymphocyte cells in a cross section of a tissue sample [10]. The system detects cell bodies in input images in two steps. First, a trained classifier computes a confidence value for each point in the image, representing the degree of believe that it is the centroid of a fluorescent cell. A so called confidence map is computed by mapping all confidence values to referential image positions. Second, the final cell positions are computed by thresholding the confidence map. To compare different classifiers, they are trained on one synthetical micrograph and applied to another. Here we compare a Local Linear Map (LLM) with a standard Support Vector Machine (SVM). The LLM was combined with different dimension reduction techniques, a Principal Component Analysis (PCA) and two different Independent Component Analysis (ICA(10), ICA(20)). To visualize the characteristics of the classifiers we show the confidence valleys of a micrograph subregion in Fig. 4. The SVM and the LLM with ICA(10) shows the most clear peaks, missing false local maxima, that can lead to false cell positions. This is reflected by the results for real micrographs (see [9] for details).

B. Learning of single cell segmentation

In another work [11], we analyze a recurrent neural network architecture for segmentation problems in biomedical computer vision. In the past, a lot of hand-tuning of the parameters was needed to obtain a good segmentation result. Thus, current research aims at the development of learning algorithms, that find a good parameterization automatically from a hand-labeled training set. The user adapts the algorithm to new image domains by supplying it with a hand marked segmentation result. Thus the biomedical researcher can use his/her primary image evaluation experience, in the adaption step and has not to learn a new skill in tuning image processing parameters. A set of synthetical micrographs of single cells have been successfully used to (a) generate labeled training sets of enough cell images and (b) evaluate the segmentation results in a quantitative way. In Fig. 5 the results of a trained network is shown. In the lower row, the four grey values of

\(^5\)The entire micrograph together with list of cell positions can be downloaded at http://www.techfak.uni-bielefeld.de/tnattkem/syncells/.

Fig. 4. The confidence maps of four classifiers (LLM with PCA, ICA(10) and ICA(20), SVM) for a synthetical micrograph are shown.
the pixels represent the segmentation evaluation result. Light grey points are true positives (cell pixels, classified as cell by the network), black points are true negatives (background pixels, classified as background). The dark grey points are false positives, the white ones are false negatives. In this study we made the interesting observation, that the generation of false negatives is not always compelled to local image parameters (e. g. caused by noise, inconsistent staining in real world micrographs), but by structural features of the network.

C. Data driven visualization of multi-parameter images

Processing of multi-parameter images is an interesting field with numerous application domains. Most applications work on multi-band satellite data in the context of oceanography or meteorology studies. Recent progresses in microscopy techniques allow the simultaneous identification of proteins in a tissue sample by recording multi-parameter stacks of fluorescence micrographs. To visualize the distribution of cellular protein patterns in such a stack, image fusion is needed to allow a fast and convenient “first look” at the data. To this end we develop an algorithm that is based on (a) the segmentation of the image into regions of homogenous protein patterns and (b) coloring the regions using self-organized maps (SOM) [12]. Step (a) is realized by a hierarchical region growing segmentation algorithm, based on an extension of the Color Structure Code algorithm [13] to multi-parameter data (MCSC). To realize (b), a SOM which is associated with a twodimensional color scale, is trained on the local parameter vectors of the image stack. Because the coloring, i.e. the SOM training, plays a crucial in the visual appearance of the data, this step must be evaluated carefully. Our approach for computing synthetical micrographs allows the customized generation of multi-parameter data sets (see Fig. 6). These are used to evaluate the dimension reduction by different algorithms. In this application we observed, that in laboratory practice several different color scales must be applied to reveal all hidden structure in the data.

V. CONCLUSION

We proposed a new strategy for image analysis evaluation and introduced an algorithm for the computation of customized benchmark data sets. The algorithm allows generation of synthetical micrographs together with ground truth labels. Using different parameters, one can easily create referential data for several real world micrograph processing problems. Our three exemplary studies clearly show the potential of the approach. A PHP-based version of the algorithm can be tested at our web site [14]. In the future, we want to add some default parameter sets to implement a limited number of standard micrograph domains, representative for our collaborative work. Nevertheless, we want to encourage other researchers to use our software for the generation of individual benchmark data sets in their particular application fields.

REFERENCES