AUTOMATED DETECTION OF INTERPHASE AND METAPHASE NUCLEI IN THE FISH IMAGES

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Abstract: The fluorescence in-situ hybridization (FISH) belongs to the most common cytogenetic methods and is widely applied in routine clinical genetic diagnostics. We are paying attention to FISH analysis of chromosomal aneuploidies – the deviations from chromosomal number. Such analysis is based on evaluation of up to several hundreds of microscopic images. Computer support for this process includes using methods of image processing and data mining. In this paper, we focus on the image processing part in more detail: first, the properties of FISH images are reviewed, then, the processing flow is outlined. Our aim is to find the interphase and metaphase nuclei and the hybridization signals contained in the image. A simple method using the raw and central moments of detected objects as measures to distinguish between the two types of nuclei is proposed.

1 INTRODUCTION

Fluorescence in situ Hybridization (FISH) is a technique used to visualize the location of specific DNA sequences within the nucleus. It belongs to the most common of molecular cytogenetic methods and is widely applied in routine clinical genetic diagnostics.

The FISH technique concerns application of a fluorescently labeled DNA or RNA probe onto a biological sample containing the target DNA or RNA from the patient (e.g. as tissue sections, separated cells, isolated interphase nuclei or chromosomal metaphase spreads). In the process of hybridization, the probe attaches to its complementary sequences on the target DNA or RNA molecule. Under the fluorescence microscope we observe the sequence with the attached probe as a strikingly luminous hybridization signal (a fluorescent dot or an area within the biological structure). Details of the principles of the fluorescence microscopy can be found e.g. in (Rittscher et al., 2008)

FISH is also a useful diagnostic tool in reproductive medicine, oncology, preventive medicine, and other medical branches – examples can be found in (Beatty et al., 2002) or (Varella-Garcia, 2003).

We are paying attention to FISH analysis of chromosomal aneuploidies – i.e., deviations from chromosomal number. These abnormalities are responsible for well-known clinically significant syndromes such as Down's syndrome (trisomy 21) or Turner's syndrome (monosomy X), Klinefeter's syndrome (XXY constitution) – see (Harper, 2004), (Roizen and Patterson, 2003), (Annerén, 2007), and (Visootsak and Graham, 2006).

To be able to conclude examination of a single patient, a set of tens or hundreds of images have to be evaluated. An automated data acquisition and processing is needed to achieve acceptable accuracy and reproducibility of results; manual processing would be time consuming and subjective.

Our paper is focused on the task of image segmentation, that is, on finding and evaluation of the objects of interest — the interphase cell nuclei, metaphase spreads and the hybridization signals. We first review the properties of images resulting from the FISH analysis in Section 2 and propose an image processing system in Section 3. In Section 4, we discuss the edge-based segmentation technique used for identification of cell nuclei and detection of hybridization signals. In Section 5, we describe the experiments and, finally, Section 6 concludes the paper.

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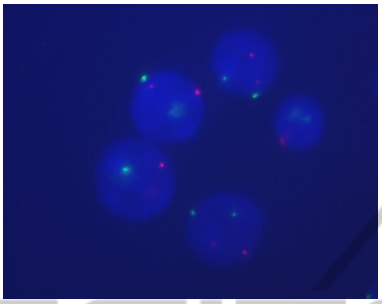
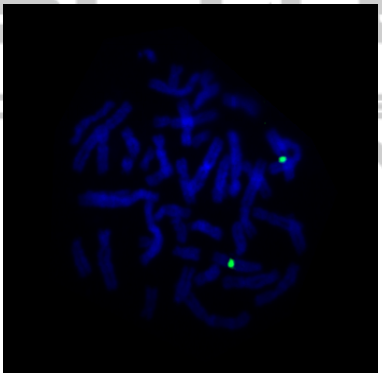
2 FISH IMAGES

Example images from the FISH analysis are shown in Figure 1. They show the interphase nucleus (Figure 1a) and the metaphase spread of chromosomes (Figure 1b), which correspond with two basic phases of the so-called cell cycle.



(a) Interphase cells



(b) Metaphase spread

Note: the images are not in the same scale. The metaphase cell image has been preprocessed to suppress the background.

Figure 1: Examples of FISH images.

The hybridization signals are — in this example — in red and green colour. Blue objects are the nuclear structures counterstained with the DAPI dye. Very often, both the interphase nuclei and the metaphase spreads are contained in the same image.

The objects of interests are characterized by the following features: the nuclei are of roughly round shape; the spread of chromosomes has ideally a roughly circular envelope. The chromosomes selves are observed as X-shaped or V-shaped bent stripes, frequently overlapping one another in the image. The hybridization signals must be located either within the interphase nuclei, or on a chromosome. The signals within a single nucleus should be clearly separated.

For detection of the chromosomal aneuploidies (cf. the Introduction section), it is important to evaluate the quality of the nuclei and hybridization signals. The rules for evaluation should be rather stringent and conservative, to eliminate dubious objects and objects

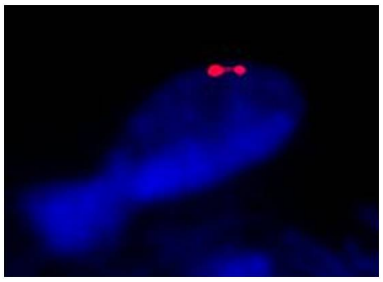


Figure 2: Interphase nucleus of an irregular shape as an example of object to be rejected from processing.

in ambiguous arrangement.

Examples of objects to be rejected from processing include (see Figure 2): interphase nuclei of irregular shape, nuclei touching or overlapping each other, metaphase spreads of significantly irregular shape, signals located in the background, etc.

For each dye, separate greyscale image is taken using filter set matching the excitation wavelength of the illuminating light and the wavelength of the light emitted by this that dye (details can be found in (Rittscher et al., 2008), Chapter 2). The resulting image is then computed from these greyscale images.

3 IMAGE PROCESSING FLOW

The processing flow of the image processing system is outlined in Figure 3, for blue-stained cells and green (or green and red) signals.

While the operations used to process the cell component and the signal components of the input image are very similar, it is advantageous to process each image, corresponding with a single filter set, separately. The reason is that the cells and the signals of each colour are in separate layers. The first operation is segmentation, used to separate the objects of interest from the background. It is followed by computation of object parameters (size, roundness, brightness, etc.), which, in turn, are used to sort out objects that satisfy the quality criteria. The results of cell processing are used to eliminate any signals located in the background.

4 IMAGE SEGMENTATION

4.1 Detection of Cell Nuclei

4.1.1 Otsu Thresholding Algorithm

For image binarization, we have chosen the Otsu algo-

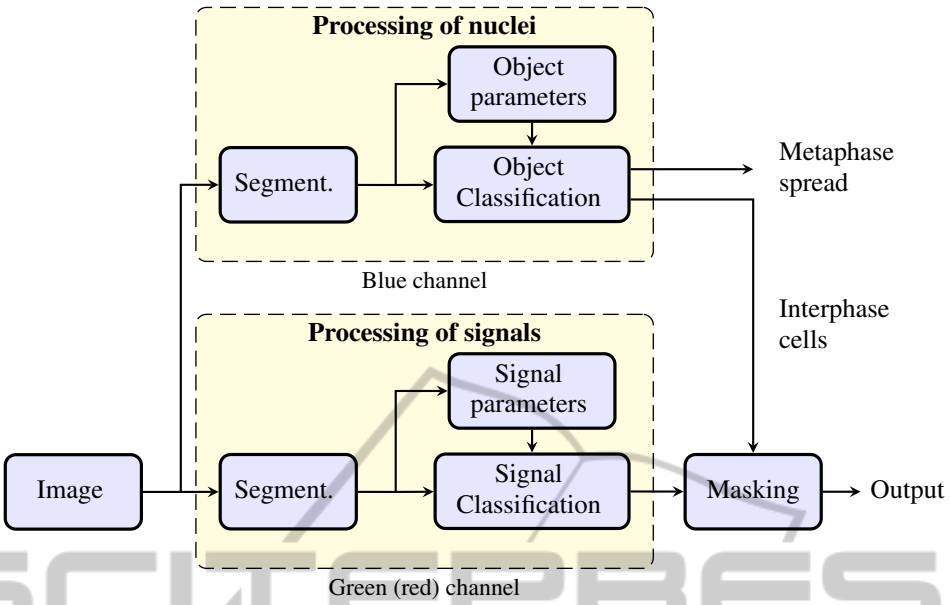


Figure 3: Processing flow of the image processing subsystem.

Figure 4: Results of the Otsu-based image binarization.

rithm, introduced in 1979 by Nobuyuki Otsu (Otsu, 1979). Using discriminant analysis, Otsu defined the between-class variance of the thresholded image as

$$\sigma_\omega^2(t) = \omega_1(t)\sigma_1^2(t) + \omega_2(t)\sigma_2^2(t), \qquad (1)$$

where ω_i are the probabilities of the two classes separated by a threshold t and σ_i^2 are variances of these classes. For bi-level thresholding, Otsu verified that the optimal threshold t^* is chosen so that the between-class variance $\sigma_\omega^2(t)$ is maximized; that is,

$$t^* = \text{argmax}(\sigma_\omega^2(t)), \;\; 1 \le t \le L, \qquad (2)$$

where L is the highest gray-level intensity value in the image.

4.1.2 Parameters of Cell Nuclei

As the next step, the eccentricity e of each object is computed, using its contour C. It is defined as the relative difference in magnitude of the eigenvalues λ_1 and λ_2 (4) of the covariance matrix of the object contour C.

$$e = \sqrt{1 - \frac{\lambda_2}{\lambda_1}}, \qquad (3)$$

$$\lambda_{1,2} = \frac{\mu'_{20} + \mu'_{02}}{2} \pm \frac{\sqrt{4\mu'^2_{11} + (\mu'_{20} - \mu'_{02})^2}}{2}. \qquad (4)$$

In this equation μ' are second order central moments defined as

$$\mu'_{pq} = \mu_{pq}/\mu_{00},$$

where μ_{pq} is the central moment of order pq defined as

$$\mu_{pq} = \sum_x \sum_y (x - \bar{x})^p (y - \bar{y})^q C(x,y). \qquad (5)$$

Only the nuclei with circular or slightly elliptical shape ($e > 0.6$) are accepted for further processing.

In addition, for each cell nucleus $\{i\}$, we evaluate further characteristics, such as the area, centroid, average intensity, standard deviation of intensity, roundness, length of contour, etc.

4.2 Detection of Hybridization Signals

Hybridization signals are detected by the same algorithm as the nuclei, but used on other layers of the input image. The fact that it is not necessary to change the segmentation algorithm to process the signals can be considered as an advantage of this approach. Illustrative results are shown in the Figure 5.

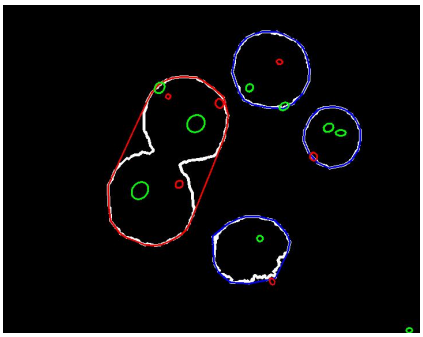


Figure 5: Detected cell nuclei with hybridization signals. Source image is depicted in Figure 1a. Note that some of the detected signals do not correspond to the real signals in the sample.

5 DESCRIPTION OF TEST DATA AND EXPERIMENTS

To test the efficiency of the proposed approach, fifty microscopic images acquired at a resolution of 720×576 pixels, containing both the interphase nuclei and the metaphase spreads of chromosomes, were used. The number of nuclei in each image ranges between 1 and 10. Most images contain metaphase spread of chromosomes and at least one interphase nucleus. This data set was divided into two equal parts, each of them with 25 images. First data set was used for nearest neighbor classifier training while the second data set for the experiments. The training set for the classifier consisted of 73 nuclei used for feature reduction. All nuclei were labeled by the features that are described in the Section 4. The features that perform well to separate both classes (interphase and metaphase), were selected manually. We have achieved the best results if contour eccentricity and length and object area were used. The classifier correctly identified nearly all (99%) interphase cells. Incomplete cells located at the image border were automatically rejected from further evaluation. Unfortunately, only 68% nuclei in the metaphase state were identified correctly.

The algorithm is sufficiently robust to detect compact spreads, such as shown in Figure 1b, but the performance decreases when the spread is not compact enough.

6 CONCLUSIONS

The paper treats automated segmentation and evaluation of images from the fluorescence in-situ hybridization (FISH) microscopic analysis.

Two problems were discussed: segmentation of the input image to cell nuclei, hybridization signals and background, and recognition of interphase nuclei and metaphase spreads. To distinguish between the two types of nuclei, eccentricity, area, and perimeter are used as the main criteria. While this approach is adequate for identification of interphase nuclei, it does not provide acceptable results for the metaphase spreads. This problem will be addressed in further research.

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