Biomedical Signal Processing and Control 8 (2013) 30–40

Contents lists available at SciVerse ScienceDirect

Biomedical Signal Processing and Control

journal homepage: www.elsevier.com/locate/bspc

# FISH image analysis using a modified radial basis function network

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ARTICLE INFO

*Article history:*
Received 22 October 2011
Received in revised form 26 April 2012
Accepted 14 May 2012
Available online 2 June 2012

*Keywords:*
FISH
Cell nucleus segmentation
Spot detection
HER-2/neu
Gene amplification

ABSTRACT

Fluorescent in situ hybridization (FISH) is an exceptionally useful method in determining HER-2/neu gene status in breast carcinoma samples, which is a valuable cancer prognostic indicator. Its visual evaluation is a difficult task, which involves manual counting of red/green dots in multiple microscopy images, a procedure which is both time consuming and prone to human errors. A number of algorithms have recently been developed dealing with the (semi)-automated analysis of FISH images. Their performance is quite promising, but further improvement is required in their diagnostic accuracy. In addition, they have to be evaluated on large FISH image data sets. Here, we present a novel method for analyzing FISH images based on cell nuclei and red/green spot modelling by radial basis functions (RBFs). Our method was compared to one of the most prominent methods reported in the literature on a large data set, comprised of 246 breast cancer cases (in total 3412 FISH images) and showed statistically significant diagnostic accuracy improvement, especially on HER-2/neu positive cases. The overall diagnostic accuracy of the proposed method is 95.93% over this data set.



## 1. Introduction

Fluorescence in situ hybridization is an established diagnostic method for gene status evaluation. It is essential in determining the status of HER-2/neu gene in breast samples, a valuable cancer prognostic and diagnostic indicator [1]. The HER-2/neu (c-erbB2) oncogene encodes the production of the HER-2/neu receptor, which is a tyrosine kinase receptor that is over-expressed in approximately 20–30% of high-grade invasive breast carcinomas. Since HER-2 positive tumors can be more aggressive, knowing that a cancer is HER-2/neu positive helps in selecting the appropriate treatment. Overexpression of the protein product of HER-2/neu gene is usually a consequence of gene amplification, in which multiple copies of the gene appear throughout the genome. Thus, it is possible to determine the HER-2/neu status, either by analyzing the numbers of gene copies in the nucleus or the amount of the related protein on the cell membrane. Fluorescence in situ hybridization (FISH) is a widely used technology to determine HER-2/neu status that allows a gene copy count. A typical FISH image of HER-2/neu is shown in Fig. 1(a). The cell nuclei have blue color, while the green and red spots map the CEP 17 and the HER-2/neu genes, respectively. The ratio of the red/green spot numbers determines the HER-2/neu status (replication) in each cell nucleus. Alternatively, the amount of protein expression can be measured directly via immunohistochemistry (IHC). There are trade-offs in choosing one of these techniques. Both techniques permit the study of small amounts of formalin-fixed, paraffin-embedded tissue and the interpretation of the findings on a cell-by-cell basis. FISH allows selective staining of various DNA sequences with fluorescent markers and, thereby, the detection, analysis and quantification of specific numerical and structural DNA abnormalities within the nuclei. It is a direct in situ technique that is relatively rapid and sensitive. No cell culture is needed in order to apply this method and results are easier to interpret than karyotype. FISH offers a more objective scoring system, based on the presence of the two HER-2 gene signals (red/green spots) present in all cells of the specimen. Its disadvantages include the high cost of each test, the long time needed for slide scoring, the use of a fluorescence microscope, the inability to preserve the acquired sample for long storage and review, and, occasionally the difficulty in identifying the invasive tumor cells [2]. In [3,4], it has been shown that this procedure is as accurate as Southern blot analysis, while allowing the measurement of the fraction of amplified cells and the intercellular heterogeneity within a given tumor cell population. On the other hand, the advantages of IHC testing include its wide availability, relatively low cost, easy and long preservation of stained slides, while the use of specific antibodies to stain proteins in situ allows the identification of several cell types that could be

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http://dx.doi.org/10.1016/j.bspc.2012.05.001

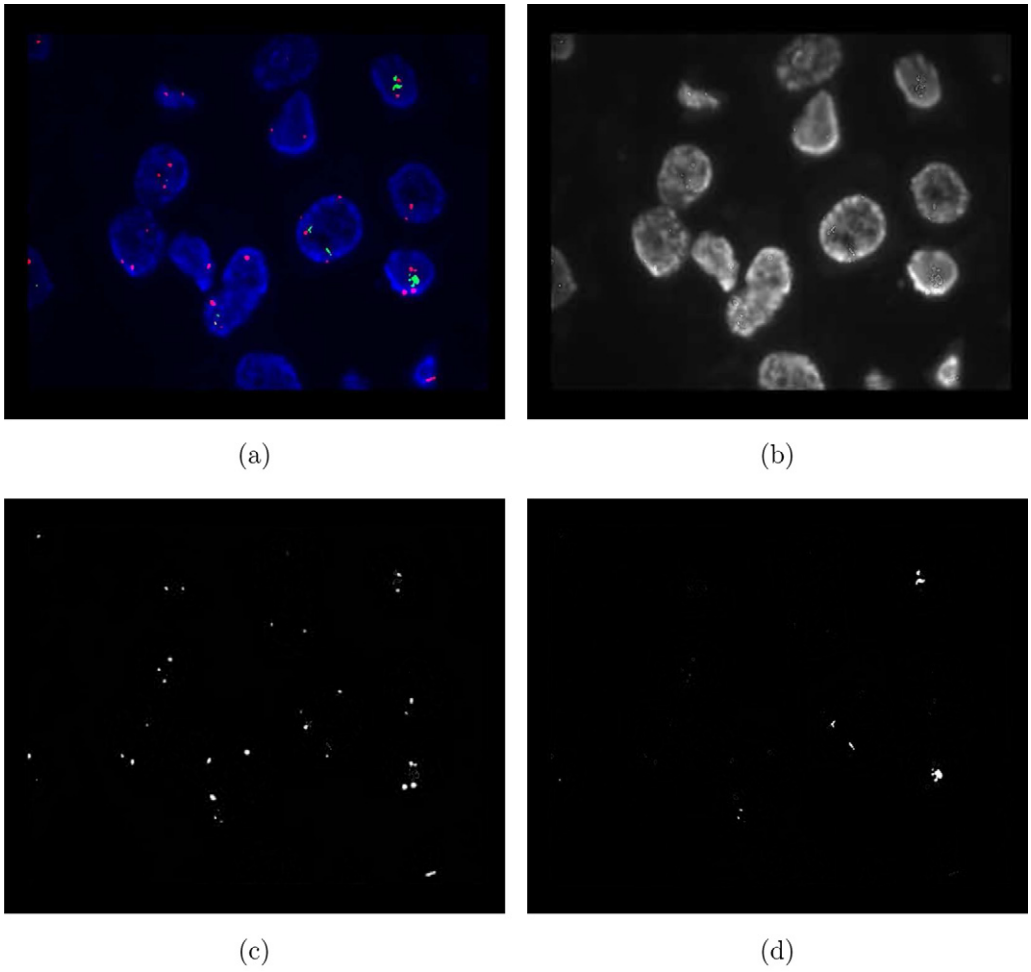


**Fig. 1.** (a) FISH image, (b) blue channel depicting all nuclei, (c) red channel spots depicting HER-2/neu gene positions, and (d) green channel dots depicting CEP 17 positions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

visualized by classical microscopy. The disadvantages of IHC include the impact of pre-analytic issues, including storage, duration and nature of system control samples and, most importantly, the difficulties in applying a subjective slide scoring system [2]. More specifically, in IHC testing, the reader must judge the degree of color change in the nucleus, against a non-standardized chart. A recent study [5] considered the accuracy, reproducibility and availability of different techniques for the evaluation of HER-2/neu status and recommended patient screening by immunohistochemistry, followed by FISH testing in cases with intermediate staining intensity (cases scored $2^+$ according to HercepTest). They suggested that the use of automated analysis may increase testing precision and predicted a wider future use of FISH analysis, as a more cost-effective technique.

Analyzing FISH images is a difficult task, since manual dot scoring over a large number of nuclei and over different tissue samples is a time consuming and fatiguing operation. Moreover, it is user-dependent in a clinical setting, since different doctors may count dots in slightly different ways, especially in ambiguities, e.g., in the case of dotted red spots or blurred spots. In case there are images containing regions with blurred spots, the pathologists assign to those regions a empirical chosen number of spots. In practice, current analysis of FISH signals is performed in a semi-automated way with the aid of image processing software, which can display the different color channels of a FISH image, as shown in Fig. 1 and apply thresholds for nuclei segmentation. One study [6] has shown strong correlation of the detection results using visual-only and semi-automated methods for evaluating the status of HER-2/neu in breast carcinomas samples. However, dot counting in a semi-automatic manner still remains an impractical procedure for a pathologist, since it requires user intervention for excluding poorly segmented, overlapping, clustered or non-relevant cells [6].

Recently, many of techniques have been proposed for analyzing FISH images targeted to a variety of genes. Most of these consist of a two-step process, namely, nuclei segmentation and spot detection. Notable examples are presented in [7], where histogram-based segmentation was performed for counting FISH signals, and in [8,9], where nuclei segmentation was performed via the ISODATA algorithm [10] and the top-hat transform was used for spot detection, followed by thresholding. In [11], nuclei segmentation was accomplished based on the ISODATA algorithm followed by the distance transform, while spot detection was based on the top-hat transform, followed by the recursive reconstruction algorithm [12]. In [13], segmentation was carried out via bi-level histogram analysis and morphological operations [14], while spot detection was performed using a watershed-like technique, called gradual thresholding. In [15,16], nuclei segmentation was performed on the blue channel, using heuristically derived thresholds and morphological operations, while spot detection was evaluated for a number of different techniques, varying from Bayesian classifiers to neural networks. In [17], nuclei segmentation was based on a variation of the watershed transform, dubbed "gradient-weighted distance transform". In [18], cell nuclei were segmented nuclei via the watershed transform, while spot detection was performed using three