



Automating Chromogenic In Situ Hybridization (CISH) Interpretation for Her2/neu marker recognition and Color Segmentation Algorithms

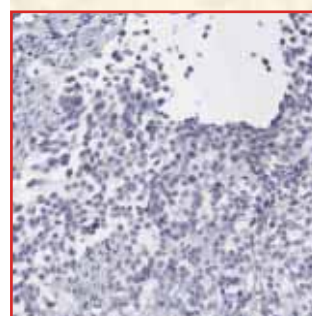
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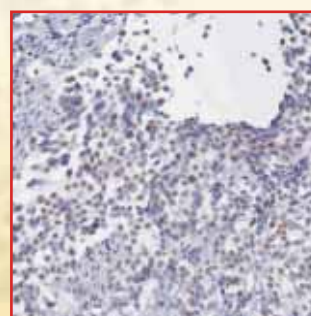
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Automated CISH Output Images

Low amplification (5 - 9 copies/nucleus)



Original Image

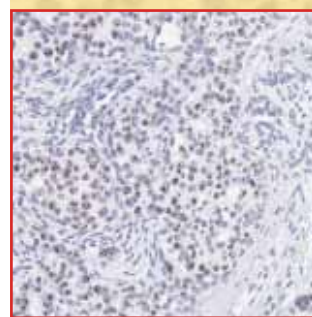


PseudoColoured Image

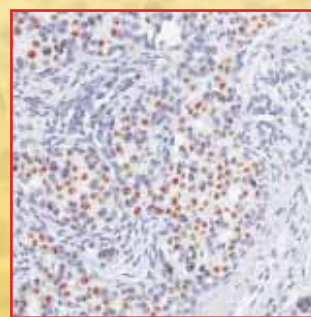


Heat Map

High amplification (More than 10 copies/nucleus)



Original Image



PseudoColoured Image



Heat Map

Background:

Detection of Her2neu amplification is an integral part of breast carcinoma diagnostics to decide therapy. Chromogenic In Situ Hybridization (CISH) is rapidly replacing Fluorescence In Situ Hybridization (FISH), the gold standard for detecting the same as a more practical alternative due to lower cost, no requirement of fluorescence microscope, ease of imaging and DNA probe detection with IHC like enzymatic (peroxidase) reaction which can be stored without fading. A major limitation in this is accurate enumeration of gene copies to decide low level Her2neu amplification (5 to 9 gene copies per cancer cell) resulting in inter-observer, intra-observer and fatigue errors; the unaltered gene status or no amplification. (one to four copies per cancer cell) and high level amplification (peroxidase positive clusters or more than 10 gene copies per cancer cell) being relatively easier to diagnose.

Materials and Method:

Any method of automated CISH interpretation should be able to identify isolated breast carcinoma tumor cells and segregate them from stromal cells. Five breast carcinomas with HER2/neu gene amplification were selected for this project.

Using light microscopy, they were assessed as 2 cases with no amplification, 2 cases with high amplification and one case with low amplification. These glass slides stained for CISH were digitized into images using a slide scanner.

Algorithms were developed for image analysis of these digital images. The area of interest in these whole slide images was found out at thumbnail level. Dense areas were considered as possible area of interest for cell

recognition. A grid of uniform size was placed on these regions with each grid not exceeding a predetermined image size of 2000X2000 pixels. Each grid was considered as field of view and indexed. The cells were validated as tumor cells based on circularity and size criteria. 40 or more isolated tumor nuclei in each field of view was considered as a requirement for enumerating copies. Automated CISH interpretation was carried out on these fields of view using novel Cell Recognition Algorithms based on Multilevel Segmentation technique and Color Segmentation Algorithms.

Multilevel segmentation technique uses two steps to identify the cells :

1. Contour tracing (identifying cells at different intensity levels) &

2. Object validation (isolated cell Identification).

Color segmentation algorithm identifies colored pixels of copies. The count of copies was made on the average size of small copies (spots). The copies in large clusters were counted by scanning each identified cell area. Image with identified copies and the image with identified nuclei were combined and the copies and nuclei were validated. Average no of copies per nucleus was estimated.

Nuclei having no copies, copies (spots) not enclosed in nuclei and overlapped nuclei were filtered out.

Results

Using unique Cell Recognition Algorithms based on multilevel segmentation technique, mathematical formula (Gaussian kernel), elongation ratio, size of nuclei, isolated tumor cells were identified and separated from surrounding stroma and stromal cells. Using Color

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Segmentation Algorithms, which detect color pixels and size of each gene copy, automated gene copy counts were enumerated and presented in the form of heat map in thumbnail image of the whole slide in different colors.

Repeated automatic interpretation of CISH slides of breast carcinoma revealed more than 98% reproducibility reducing inter as well as intra-observer variation and fatigue errors. In the tumor with normal Her2/neu copy number as assessed by light microscopy, the average number of copies per nucleus measured using our algorithm was

Discussion

The present experiment used automation for the detection of CISH for detecting HER2neu gene amplification in breast carcinomas as a prototype. These preliminary results show that this is a feasible approach. With the use of Cell Recognition Algorithms and Color Segmentation Algorithms, the automated interpretation of CISH for other DNA probes can be investigated by imaging techniques. This method can thus help reduce subjectivity and ease the interpretation of CISH staining results technology generically.