분자 세포 유전학 기법에 응용되는 영상 처리 기술 [1]

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Image Analysis Algorithms for Comparative Genomic Hybridization[1]

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요약

Comparative Genomic Hybridization (CGH)은 세포 내 특정 DNA 서열 이상을 염색체상에 보여주는 중요한 분자 세포 유전학 기법이다. CGH 기법에서는 세포 분열 중기의 염색체에서 준비한 형광 비율 영상의 정량적 분석을 위해서 Digital 영상 처리 기술이 쓰여야 한다. 본 논문에서는 최근 연구 개발된 영상 처리 algorithm 들이 어떻게 CGH 기법에 쓰이는 지를 소개하려 한다. 각 염색체의 형광 비율 profile 를 평균하기 위해, 염색체 영상의 이원화, 염색체 영상 뼈대 변환 (skeletonization), 뼈대 정보의 변수화와 영상 명암의 재추출을 통한 굽은 염색체 영상 펴기 등이 연구되었다. 개발된 algorithm 들은 바이오메드랩 사의 ChIPS 핵형 정렬 시스템에 구현했다.

Abstract: Comparative genomic hybridization (CGH) is an important molecular cytogenetics technique that maps abnormal copy number of specific DNA sequence of the chromosome. CGH is based on quantitative digital image analysis of ratio images from fluorescently labeled chromosomes. In this paper, we would like to introduce how recently developed image analysis algorithms are used for CGH techniques. To average the ratio profile of each chromosome, binarization, skeletonization, and stretching of chromosome images have been studied. Developed algorithms have been implemented in the karyotyping system ChIPS commercially developed at Biomedlab Co. Ltd.

Key words: Comparative Genomic Hybridization, Image analysis, Fluorescence microscopy, Chromosome.

INTRODUCTION

Comparative genomic hybridization (CGH) is a molecular cytogenetics technique that visualizes *in situ* increased or decreased copy number of specific DNA sequence in chromosomes. The copy number can be represented as a profile along the length of a chromosome that is calculated from the ratio image of the chromosome labeled with two different fluorochromes. Chromosomal aberrations are detectable through averaging profiles of the chromosome sampled from many metaphase images. Sophisticated digital image analysis techniques are often required for the quantitative analysis of CGH images.

The focus of this paper will be on the image analysis part of CGH technique. We describe crucial steps in the evaluation process of the CGH signals by using artificial chromosome images and present an analysis result using images obtained from a clinical sample.

MATERIAL AND METHODS

1. CGH slide preparation and Image acquisition

CGH slides were kindly provided by Dr. Soo Kyung Choi in Samsung Cheil Hospital and Women's Healthcare Center, Seoul. The experimental set up for acquiring CGH fluorescence images was similar to what have been described previously in detail[1].

2. Image analysis algorithms for CGH experiments

A set of fluorescence images was artificially generated to test the performance of a stretching algorithm and the accuracy in extracting the fluorescence ratio profile (Fig. 1). Fig. 1a shows a counter-stained image that was used for generating chromosome mask. Fig 1b and c

represent two fluorescence images that were used for calculating the ratio profile of test DNA fluorescence (Fig. 1b) versus control DNA fluorescence (Fig. 1c). In Fig. 1b, the bright bands were assigned as 125, the dark bands as 75, and the rest of chromosomes as 100, whereas whole chromosomes in Fig. 1c were assigned as 100.

Fig. 2 represents a simplified procedure of image analysis for CGH techniques. Fluorescence ratio images (Fig. 2a) were obtained through interactive thresholding of chromosome images, semi-automatic correction of optical pixel shift, background subtraction,
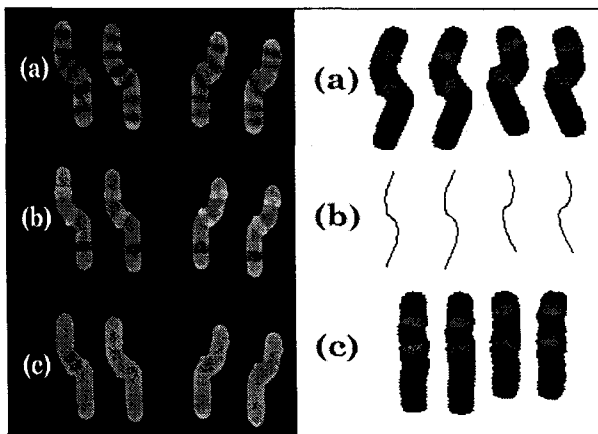

Fig. 1. Three artificial fluorescence images of two pairs of homologous chromosomes.

Fig. 2. Image processing procedures for CGH techniques. Ratio image (a), chromosome skeleton (b), and stretched ratio image (c).

ratio calculation, and image rotation[2]. Skeletons (Fig. 2b) were calculated for each chromosome using a thinning algorithm[3]. Finally, ratio images were stretched based on parameterized skeleton pixels and resampling of ratio data along the curve that equally divided the vertex angle of each skeleton pixel (Fig. 2c). The ratio profile was calculated by averaging the ratio data of each row of the stretched ratio image. The average ratio profile representing specific chromosome was calculated by averaging the ratio data of two homologous chromosomes and by normalizing the length of the profile to a predetermined length.

3. Analysis of a clinical CGH sample

A sample set of metaphase chromosome images, obtained from a patient known to have increased DNA sequences of chromosome number 8 and 18, was also tested to confirm the performance of developed

algorithms. Each chromosome was manually karyotyped and chromosome images were processed with the same algorithms described in the previous section.

RESULTS AND DISCUSSION

Ratio profiles were displayed next to the ideogram of the chromosome (Fig. 3). Two profiles showed the exact ratio values of 1.25 at the bright bands, 0.75 at the dark band, and 1.0 at the rest of the chromosome. Chromosome 8 and 18 from the sample set showed significantly increased ratio values ($p<0.05$) and the rest of chromosomes were within the normal range of ratio values (data not shown). Future development of algorithms would involve automatic karyotyping of chromosomes and fully automatic correction of optical pixel shift.

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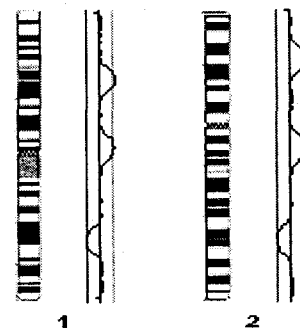
Fig. 3. Ratio profiles displayed next to the ideogram of chromosome number 1 and 2. The central line represents the balanced state defined as the mode of the histogram of the average ratio profile for all chromosomes. The left line was 0.75 and the right line was 1.25.