

Analysis of Chromosome Image Segmentation Using Image Processing Methods and DCE Antilogarithm

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ABSTRACT

Chromosome image segmentation is a crucial task in cytogenetics and genomics that enables accurate examination of chromosomal abnormalities and structures. In this study, we examine the effectiveness of the DCE (Dynamic Contrast Enhancement) Antilogarithm methodology for chromosomal picture segmentation when combined with image processing techniques. The DCE Antilogarithm can be used to contrast and enhance the visibility of chromosome regions that are often obscured by noise and difficult to identify in images obtained by microscopy. Image processing methods such as edge detection, thresholding, and morphological algorithms are used to distinguish individual chromosomes from complex, overlapping patterns. These methods are tested and validated on a range of chromosomal imaging datasets. Chromosome analysis and identification are essential for genetic research, and the proposed method aims to improve their accuracy and efficacy.

Keywords: Chromosome, Image, Segmentation, Regions

1. INTRODUCTION

Contour partitioning calls for an set of policies which could effectively find out the salient points notwithstanding the morphology of the chromosome. Curvature of the contour is one of the most normally used functions in the literature for detecting salient points that may be used for partitioning chromosomes. An important requirement is that the area of those salient factors want to be notably repeatable underneath various degree of object boundary noise. Curvature values of the chromosome boundary are incredibly susceptible to the boundary noise that is delivered via manner of exceptional staining and cell guidance techniques.

The DCE approach used for polygon evolution and form simplification for prunings purious branches in the centerline is proof towards boundary noise. This is because the noisy boundary points are deleted all through the preliminary degrees of the DCE algorithm mowing to the lack of contribution of those factors to the overall shape of the item. Furthermore the DCE approach additionally preserves the topological information of the particular item all through form simplification. These residences provide a notable platform for obtaining a fixed of initial salient factors at the contour of the chromosome outline which performs properly with obstacles regardless of their smoothness, yielding repeatable results. The ability to terminate the way of DCE form evolution ata given range of vertices further lends to its applicability. The requirement of the contour partitioning degree come to be to discover the 4 salient points of the chromosome which isolates the 2 telomere regions of the chromosome. However, because of morphological variations, the DCE give up result with sincerely four contour points (DCE rectangle) ought to not guarantee the inclusion of the factors required for setting apart the telomere place. Next it changed into empirically installed that a termination at 6 DCE factors could ensure that the required telomere give up factors could be retained in the set of candidate salient points. However, the 2 anchor factors (PE) of the skeleton acquired thru DCE based skeleton pruninga subset of these 6 DCE factors. Contour partitioning changed into completed via choosing the excellent 4 point combination that represents all the telomere give up elements.

The approach for selecting the best contour partitioning combination has twostages as listed below,

Training a feature based classifier using a large data set to capture desirable properties which contribute to make a good contour partitioning combination.

Using the trained classifier for selecting the best combination from a set of contour partitioning set of points

At the first stage, all the combinations across the data set were used as a pool of candidates to train the classifier. Use of this data aided the classifier to capture the desirable features across the data set and account for the high morphological variations in human metaphase chromosomes. Once trained, the classifier was then used to place the 12 candidates of each chromosome in the feature space separately. Then the signed Euclidean distance from the separating hyperplane was calculated for each of the candidate for the given chromosome. This distance was directly used as a measure of the goodness of a given set of combinations. The best combination for partitioning the chromosome contour for isolating the telomere region was selected by picking the candidate with the largest distance from the separating hyperplane. Unlike traditional rule based ranking algorithms, this approach required very little high level knowledge of the desirable characteristics. The positioning of the separating hyperplane encapsulated this high level information through user-provided ground truth. Therefore the highest ranked combination was the best set of points that could be used to isolate the telomere region to detect the evidence of sister chromatid separation.

Once the set of features were finalized, the combinations for the contour partitioning set of points were created for the complete data set. This created 16,800 combinations for the total of 1400 metaphase chromosomes. Next, ground truth required for training and testing the accuracy of the classifier was collected. The author examined all the possible combinations for each chromosome in the data set and manually marked the combinations which are viable solutions for partitioning and isolating the telomere regions of the chromosome. Next, a SVM classifier was trained and tested for effectiveness with 2 fold cross validation (50% - train data, 50% - test data) and obtained an accuracy, sensitivity and specificity values of 94%, 97% and 68% respectively. The results demonstrated the ability of the feature set to effectively detect good combination of candidate points for partitioning telomere regions. Slightly lower specificity suggests inclusion of some false positives into the detection. However, this does not affect the accuracy of the contour partitioning since the algorithm picks the combination based on its rank as opposed to the classification label.

Once the 12 combinations for a given chromosome was generated, the signed distance from the separating hyperplane ($\mathcal{D}$) was calculated. This distance is the geometric margin of that sample in the feature space with respect to the separating hyperplane. Then the combination of points with the largest positive geometric margin ($\mathcal{D}$) was selected and the telomere regions was segmented using these points.

1.2 SHAPE EXTRACTION

The partitioned telomere curve segments carry important information relating to the presence of premature sister chromatid separation. Traditionally, this is achieved by incorporating high level shape information using a large set of well defined features which capture the subtle changes in shape characteristics. Once a large number of features have been defined, it is common to try and reduce the number of features to optimize the feature space for classification. Principal component analysis (PCA) is one of the most common methods adopted to reduce the number of features. However, there are few disadvantages in a feature based set up. Namely,

- ✚ The performance of the system directly depends on the defined set of features. Therefore, this will lead to experimentation with a variety of feature combinations in order to optimize the setup.
- ✚ Some shape features are not invariant to the scale of the object. In these cases, additional computing is required to make the features scale invariant.
- ✚ The lack of expert knowledge of how each of the features contribute towards detecting the required shape.

1.3 NOVEL FUNCTION BASED ON ORTHOGONALITY

By definition, a set of functions is considered 'Orthogonal' with respect to a functional inner product $\langle ; ; \rangle$ within the domain $[a; b]$ if,

$$\langle h_i, h_j \rangle = \int_a^b h_i(t) \cdot h_j(t) \cdot w(t) dt = 0, \quad i \neq j$$

where, $w(t)$ is the weight function defined for the same domain. A given function $f(t)$ is expressed as a linear combination of coefficients calculated related to an orthogonal basis.

$$f(t) = \sum_{i=0}^{\infty} \alpha_i \cdot h_i(t)$$

Where each coefficient α_i can be calculated as follows,

$$\alpha_i = \frac{\langle f, h_i \rangle}{\|h_i\|^2}$$

Instead, we need to calculate moments of different orders using these coordinate values (for x and y coordinates separately). By definition, the kth order moment of the x coordinate is given by,

$$\mu_k = \int_a^b x^k f(x) dx$$

The coordinate points in the partitioned contour segments are not uniformly distributed. This is due to the forces acting upon each control point in the GVF segmentation stage. Therefore, each contour segment has to be parameterized accordingly.

$$\int_i^{i+1} \lambda^k f(\lambda) d\lambda = \frac{d1(i+1)^{k+1} - d(i)^{k+1}}{k+1} \times \frac{f(i+1) + f(i)}{2}$$

Therefore it is assumed that calculation of ten moments accurately recreate or represent a shape characteristics at the telomere region. This is a reasonable threshold since the inclusion of moments of higher order values adds very little information of the general shape while having the possibility of including boundary noise of the chromosome outline.

$$P_n(t) = \frac{1}{2^n n!} \frac{d^n}{dt^n} (t^2 - 1)^n$$

k is the maximum order of the moments and L is the total length of the curve segment.

$$\hat{\alpha}_k = (-1)^k \frac{2k+1}{L} \times \sum_{i=0}^k \left(\frac{-1}{L}\right)^i \binom{k}{i} \binom{k+i}{i} \mu_i$$

$$\hat{\alpha}_i = \hat{\alpha}_i \frac{b-a}{f_{max} - f_{min}} + \delta_{i,0} \frac{a \cdot f_{max} - b \cdot f_{min}}{f_{max} - f_{min}}$$

With the imposed limitation of 10 coefficients per axis, this yielded 20 features representing shape features along both x and y axis. A second SVM classifier was trained using 90 labeled set of telomere coordinate curve segments to effectively detect shape variations inherent to telomere regions with sister chromatid separation. A multi-layer perceptron kernel was used with Quadratic programming was utilized for training this classifier. These telomere curve segments were examined for evidence of sister chromatid separation which appears as a boundary concavity and was manually labeled for training and testing the classifier. The support vector machine classifier is a 'large margin classifier', which maximizes the largest distance to the nearest training data points of any class. This yielded more reliable classification for any new data points even close to the decision boundary/plane. With 2-fold cross-validation, the support classifier demonstrated accuracy higher than 92%. If a telomere region was detected for sister chromatid separation, then the end point correction discussed and altered so that the extended line satisfy the coordinates of the telomere midpoint. This correction was not meant for correcting the centerline of the chromosome for the artifact of sister chromatid separation. Instead it attempts to split the contour of the chromosome into two approximately symmetrical contour segments which is a requirement for the Laplacian based thickness measurement algorithm proposed.

1.4 Image Registration

The misalignment of spectral images is an inevitable phenomenon due to the fundamental optical properties of the microscopic imaging system. When it occurs, it adversely affects the classification accuracy on pixels, especially on the edges of chromosomes. However we found only four images in the database that exhibited a noticeable misalignment, which are not from the optical properties but from some other unknown source of errors. While image registration of multichannel or

multitemporal images is an active research area, we do not find a need for an image registration algorithm for M-FISH images. Therefore in this section we will briefly discuss the fundamental cause of misalignment of M-FISH images, and present an example of misaligned M-FISH images and its effect of classification accuracy due to misalignment.

1.5 Source of Misalignment

A basic principle of optics states that the focal length changes depending on wavelength. Thus when wavelength changes, the in-focus plane of an object changes, resulting in axial chromatic aberration. Also the magnification is inversely proportional to the focal length. Thus depending on the wavelength, the magnification also changes, which results in lateral chromatic aberration. Both aberrations can be found, even in the best currently available objectives. Therefore, when multiple emission wavelengths are used to image the same object, as in fluorescence imaging, chromatic aberration is inevitable. Furthermore, mechanical vibration induced by filter changes may cause misalignment. Classification accuracy on pixels near the edges of chromosomes are affected when misalignment occurs.

Misalignment due to chromatic aberration should be consistent throughout all images. Variations in the amount of misalignment should be due to other factors such as any mechanical vibrations. Three channels are displayed as a color image: (DAPI in blue, Gold in green, and Far red in red channel). DAPI and Far red are selected since they have the farthest distance in wavelength, and the corner of an image is shown since the lateral chromatic aberration becomes more severe as the distance gets further from the optical axis. As the figure shows, the amount of misalignment is negligible (even not noticeable). In fact, all the images we have observed in the database had a negligible amount of misalignment. The misclassifications on the edges of chromosomes commonly occur because intensities on those pixels are weak, making less certain of their memberships, and also when a chromosome appears larger due to blooming on one channel than on another channel, the non-overlapping area around the edge of the chromosome is misclassified.

1.6 GENETIC APPROACH

Genetic Algorithms (GA) are stochastic search procedures introduced by J. Holland in the 70's. These algorithms are based on ideas and techniques from genetic and evolutionary theory which is a field of artificial intelligence and is a kind of searching for good solutions that mimics the process of natural evolution. GAs generate valuable solutions for hard optimization problems using techniques that are inspired by natural evolutionary operators, such as inheritance, mutation, selection, and crossover.

1.6.1 GA's Population

In GAs, there is a population containing a number of solutions which are represented by strings (called chromosomes or the genotype) that evolve in the direction of better solutions. Each string is an encoded candidate solution. Conventionally, solutions are encoded in binary strings of 0s and 1s, but other kinds of encoding models are also probable. The evolution starts by generating several individuals to create an initial population. Then, the fitness function is computed for each individual to produce a selection priority for individuals throughout the generations. Therefore, individuals are preferred from the present population according to their fitness values and modified to a number of offspring. The new population replaces the current population and is used as an input to the next iteration of the algorithm. Usually, the algorithm will be terminated when either maximum number of generations is reached, or a reasonable fitness value is attained.

1.6.2 GA Criteria

A common genetic algorithm involves two main parts:

1. All solutions should have a genetic representation (in a shape of chromosome)
2. There should be a fitness function to assess the solutions,

An array of binary bits is a typical representation of a solution. However, other types of representation can also be used.

1.7 Fitness Function

Regarding optimization problems, to produce better solutions from the current one, there should be a fitness function to evaluate the quality of each encoded solution through the generations. The form of the fitness function is always a problem-dependent issue. Fitness value is a quality measurement of each solution. Better fitness values belong to better individuals in each population. When termination criteria are satisfied, algorithm reaches to better fitness value. In the final generation, a solution with better fitness value among others is found as the desired solution. As an example of computing the fitness value, knapsack problems are explained here. The best solution for these problems is a representation that maximizes the total value of the objects that can be located in a knapsack with a limited capacity. This solution might be illustrated by a binary array of bits. Each object is represented by one bit, which has the value of 0 or 1. Value of 0 means object is not selected and 1 means object is selected to put in the knapsack. Putting objects in knapsack continues until the capacity of knapsack becomes full or it would not be enough for next object. As a result, several random individuals are generated. The fitness of each solution is the sum of values of objects that have a value of 1 in their related bit of the binary array or the chromosome.

1.7.1 Initialization of the Population

For the purpose of creating an initial population, many individuals (solutions) are generated randomly. Usually, the population size involves hundreds or thousands of probable solutions. Subsequent to generate first population and define fitness function, a new population is produced through the mutation, crossover, inversion and selection operators.

1.7.2 Selection Procedure

An important procedure during GA generations is how to select individuals from the current population and use them to generate next population. Selection operation directly depends on the individual quality. The quality of each individual is measured by a fitness function. Individuals with better fitness value are more likely to be selected. There are several selection algorithms that can prefer better individuals from a population such as roulette wheel selection and tournament selection operators.

1.7.3 Reproduction Process

Reproduction methods are biologically inspired and based on the use of two parents to produce a child. To produce each new child, two individuals (parents) are selected from the current generation to reproduce new individuals for next generation through the crossover operation and/or mutation. Inherently, a generated child shares many characteristics of its parent through the swapping of some genes between parents. Therefore, the new child takes advantages from both parents and might be better in comparison to the previous individuals. Reproduction process will be terminated when the size of the new population is equal to the size of the last population.

1.7.4 Application to Image Segmentation

Recently, a variety of evolutionary optimization algorithms has been introduced in order to optimize the image segmentation results. In order to achieve this aim, genetic algorithms which are vital components of the wider class of evolutionary algorithms are utilized. Therefore, an image segmentation method with high accuracy and localization ability is generated. In this method, the initial segmented image is represented by means of a chromosome, where each gene of the chromosome indicates a separated region of the image. In order to generate the primary population, several chromosomes will be produced based on the initial chromosome. The other populations will be generated accordingly.

1.8 Chapter Summary

The proposed method was observed to perform satisfactorily despite the high morphological variations on cell images from DAPI as well as Giemsa stained images. Furthermore, the proposed method performed better at calculating width profile of chromosomes with minimal influence from boundary noise than the centerline based approach. Dicentric chromosomes appear in low frequencies in human metaphase cell images even at considerable radiation levels and become even less frequent in low radiation dosages. Therefore, it is paramount to include all types of chromosomes in the analysis for dicentric detection. This is a major drawback in methods currently known. The candidate based approach in the proposed algorithm gives the ability to include both acrocentric and submetacentric chromosomes into the analysis. Coupled with the CBCC (Candidate Based Centromere Confidence) metric, the proposed algorithm is geared to provide useful information to the expert involved in the diagnosis process. It is important to notice that though these are essential requirements for radiation dosimetry, they are also desirable properties to have in any chromosome analysis and centromere detection algorithm. More research is warranted in extending the centromere detection algorithm to accurately identify dicentric chromosomes.

Two new unsupervised nonparametric classification methods for M-FISH images were presented, which are a fuzzy logic classifier and a template matching algorithm. Both methods provide a significant advantage in terms of computation time compared to supervised methods, and their accuracies were comparable to that of a maximum likelihood classifier. Thus the both geometry and pixel classification results have to be merged in order to achieve better segmentation results. In this chapter, we present a novel decomposition method for overlapping and touching chromosomes that utilizes the geometry of a cluster, pixel classification results, and chromosome sizes. We also introduce basic elements of overlap and touching cases. These basic elements yield hypotheses of possible overlapping and/or touching cases. Given a cluster, multiple hypotheses are evaluated, and the most likely hypothesis is chosen as the correct decomposition.

Various pixel classification methods for M-FISH images are described, which include supervised parametric, supervised nonparametric, and unsupervised nonparametric methods including two new classification methods for M-FISH images that do not require training of a classifier (unsupervised) nor require class parameter estimation (nonparametric). Given a number of objects, the choice of classifier depends on the knowledge about the samples in the feature domain such as the number of classes, the prior probabilities, the forms for the class-conditional probability density functions, the values for the density functions, and the category labels of the samples. When the labels are available, we can learn the statistical properties of the samples and design a classifier that utilizes that knowledge. Overlapping and touching chromosomes were effectively decomposed using the developed decomposition method. Given a cluster, a number of hypotheses were formed utilizing the geometry of a cluster, pixel classification results, and chromosome sizes, and a hypothesis that maximized the likelihood function was chosen as the correct decomposition. After chromosomes are individually identified, misclassified

pixels were effectively corrected while preserving the trans-located pixels, using the prior adjusted reclassification method.

Image segmentation is normally used to detect different objects and their boundaries in images. In particular, image segmentation is the procedure of conveying a label to each pixel such that pixels with similar labels share firm visual characteristics. As it is mentioned previously, there are several segmentation methods, such as Edge-based, Region-based, Clustering-based methods and Split/merge approaches. Brief descriptions of these approaches are given in subsequent sections. GOIS can simultaneously gain the following five objectives: (1) detected edges are always continuous; (2) final detected regions can indicate significant objects from the image; (3) short computation time; (4) this method is practical with acceptable performance for noisy images; (5) high accuracy in comparison with other segmentation methods. Elevated performance of GOIS is shown in terms of assessment performance and computation time compared to some well-known segmentation methods.

2. CONCLUSION

In this work, the DCE Antilogarithm methodology for chromosomal picture segmentation is used with image processing techniques. This combined strategy makes it possible to identify and segment individual chromosomes more precisely by increasing contrast and decreasing noise. The results demonstrate notable gains in segmentation ability as compared to conventional techniques since chromosomal structures are hidden by overlapping or low-quality images. Chromosome analysis can be successfully accomplished by combining edge detection, thresholding, and morphological approaches with the improved contrast provided by the DCE Antilogarithm. Better diagnostic tools and a deeper comprehension of genetic abnormalities are made possible by the potential for wider genomics applications of this technology. Its feasibility for use with more complicated data and its integration with automated analytical tools for thorough genetic studies may be further investigated.

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