Wavelets meet genetic imaging

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ABSTRACT

Genetic image analysis is an interdisciplinary area, which combines microscope image processing techniques with the use of biochemical probes for the detection of genetic aberrations responsible for cancers and genetic diseases. Recent years have witnessed parallel and significant progress in both image processing and genetics. On one hand, revolutionary multiscale wavelet techniques have been developed in signal processing and applied mathematics in the last decade, providing sophisticated tools for genetic image analysis. On the other hand, reaping the fruit of genome sequencing, high resolution genetic probes have been developed to facilitate accurate detection of subtle and cryptic genetic aberrations. In the meantime, however, they bring about computational challenges for image analysis. In this paper, we review the fruitful interaction between wavelets and genetic imaging. We show how wavelets offer a perfect tool to address a variety of chromosome image analysis problems. In fact, the same word "subband" has been used in the nomenclature of cytogenetics to describe the multiresolution banding structure of the chromosome, even before its appearance in the wavelet literature. The application of wavelets to chromosome analysis holds great promise in addressing several computational challenges in genetics. A variety of real world examples such as the chromosome image enhancement, compression, registration and classification will be demonstrated. These examples are drawn from fluorescence in situ hybridization (FISH) and microarray (gene chip) imaging experiments, which indicate the impact of wavelets on the diagnosis, treatments and prognosis of cancers and genetic diseases.

Keywords: Chromosome analysis, microarray, wavelet, genetic aberrations

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1. INTRODUCTION ON GENETIC IMAGING

Cancer is the second leading cause of death in the US, behind heart disease. In the US, one of every four deaths is from cancer. About 20% of cancers can be revealed by genetic or chromosomal aberrations. Many diseases, cancers and birth defects are a direct result of chromosomal or DNA segments aberrations. Geneticists can recognize and identify many of the chromosomal abnormalities or DNA copy number changes by examining chromosomes using a microscope in a cytogenetic or molecular cytogenetic laboratory. Chromosome abnormalities can be extremely complex [2]. Two basic types are *numerical* and *structural* abnormalities. Numerical abnormalities involve the loss and/or gain of a whole chromosome or chromosomes and can include both autosomes and sex chromosomes. For example, the patient with Downs syndrome has an extra copy of chromosome 21. Structural abnormalities involve changes in the structure of one or more chromosomes. They can be incredibly complex and the three of the more common types are *deletions*, *inversions* and *translocations.* These chromosomal aberrations are correlated with phenotypic abnormalities for cancer and genetic research, diagnosis, and prognostic.

For over forty years, cytogenetics and digital imaging, two seemingly unrelated fields, have formed an intimate partnership due to a subtle symbiosis. Digital imaging has contributed to cytogenetics instrumentation that reduces the workload in clinical labs and produces quantitative data for both research and diagnosis [3, 4]. At the same time, cytogenetics has posed novel problems that prompted the development of a broad array of techniques useful in fields far beyond genetics. The parallel and interacting histories of these two fields can be traced back to the early of 1980s. Breakthroughs in one led to advances in the other throughout their development. The last few decades have seen significant progresses as a result of their combination or cross-fertilization. Genetic imaging techniques such as chromosome G-banding and molecular florescence in situ hybridization (FISH) [4], spectral karyotyping (SKY), comparative genomic hybridization (CGH) [5] and multiplex fluorescence in situ hybridization (M-FISH) [1] have been developed.

The advent of microarray techniques and the human gene mapping project open new avenues for cytogenetics [6]. A number of high resolution probes have been developed, such as the single copy probes for fluorescence in situ hybridization (scFISH) [8] and high resolution BAC microarray CGH (<200kb) technique [7]. However, these high resolution probes give rise to specific problems. Because the high densities of these probes, they produce vast amount of data for processing. Major challenging problems include:

- The throughput -- The fully automatic diagnosis is still the bottleneck of many research and clinical genetic laboratories.
- The sensitivity -- The probability of yielding positive results when a genetic aberration is present has not reached the desired level.
- The resolution -- The ability of the imaging algorithms to resolve the subtle and complex chromosome aberrations remains limited. The use of image processing is somewhat lagging behind the development of high resolution genetic regents.
- The reproducibility -- The imaging systems are not always reliable to produce the same output when genetic experiments are performed at different times.

These requests are particularly challenging for high resolution probes. For example, to map a common site of chromosome breakage in 50 patients using the scFISH approach might require 10 probes, the equivalent to 1000 slides, and each slide could generate up to 2000 raw images (2 Gb). This vast amount of data necessitates the need for high throughput processing. Despite the availability of many commercial analysis software packages, full automation of the diagnostic procedure remains a problem. In this challenging area of genetic image analysis, the development on the image processing approaches is lagging behind the industrial development.

2. RATIONALE OF WAVELET APPROACHES

Wavelets offer a perfect tool to address the genetic imaging problems. By transforming chromosome images into wavelet bases, the extraction of salient image features is facilitated. In fact, wavelets have an interesting connection with the discovery on multiresolution structure in genetics.

It has been recognized in genetics that chromosome banding patterns constitute the most distinct features for chromosome classification. These alternating light and dark patterns are gene rich and poor regions of a genome. When metaphase chromosomes are stained in different ways and examined under microscope, a multiscale structure of the bands is seen. Figure 1 shows an example of chromosome banding patterns, which have multiple resolutions [2]. This is strongly consistent with the concept of wavelets, which decompose a signal in multiple resolutions, revealing the finer details of the signal at high resolutions. In the nomenclature of cytogenetics, the exact same word "subband" has been used to name different levels of chromosome band resolutions as that in wavelet theory [2]. Therefore, it could be hypothesized that the human genome sequences might be encoded and thus revealed by wavelet bases. In fact, work has been conducted on the use of wavelets to characterize the DNA contents in different regions from human genome sequencing data [12, 21, 22]. The application of wavelets to chromosome images offers distinct advantages.

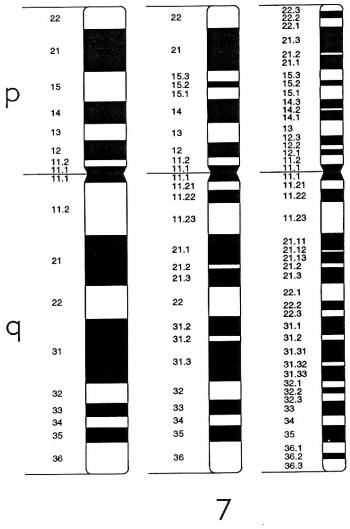

Fig.1 The multiresolution structure of chromosome bands. The dark bands are AT rich while light band are GC rich. Three different levels of band resolution for chromosome 7 are shown, which are resulting from different chromosome preparations [2].

- Wavelets provide compact information representation. The multiresolution representation of chromosome image using wavelets has the intrinsic ability to compact the energy (i.e. structure) of a chromosome banding signal into a few coefficients, thus facilitating subsequent data analysis. The sinusoids, Gaussians and Weighted Density Distribution (WDD) functions have been commonly used for representing chromosome banding patterns. It has been indicated that wavelet bases can achieve a greater chromosome classification rate than these commonly used basis functions [20].
- Wavelets facilitate the extraction of important geometric features. Our work has shown strong evidence that chromosome banding patterns can be revealed by means of wavelets and thus can be used for enhancement of these features [9].
- Wavelet analysis offers computational advantages. The fast filter bank algorithm developed in wavelet theory enables us to design efficient image processing algorithms.

Extensive applications of wavelets to other biomedical imaging problems have been studied. However, to our best knowledge, the application of wavelets in chromosome image analysis has been limited. Wavelet approaches have strong potential to make a breakthrough in solving the computational challenges faced by genetic imaging.

3. APPLICATION OF WAVELET APPROACHES TO GENETIC IMAGING

We review a variety of projects that we have been doing at the University of Missouri-Kansas City (UMKC) and Advanced Digital Imaging Research (ADIR), LLC with the collaboration of medical geneticists from Baylor College of Medicine and Children's Mercy Hospital. These projects involved several imaging methodologies at the cellular level, including chromosome G-banding, FISH, M-FISH, CGH and microarray imaging. Wavelets have played a significant role in these projects. In particular, we have used a special family of spline wavelets [12], which have good properties such as computational simplicity and translation invariance. Due to space limitation, we omit the details of the algorithm but give the references for each project.

3.1 Optimization of optical microscope system performance for accqusition of chromosome images

The automatic acquisition and visualization of FISH probes or G-strained chromosomes for a large number of cells is performed with a computer driven microscope. In the optical system, all components and software modules are optimized for speed and quality. High resolution is obtained by means of high numerical aperture (NA) of the objectives typically combined with a cooled high resolution digital CCD camera. The performance of the optical system depends on many factors, such as the type of objective correction, the refractive index of the embedding medium, the emission

wavelength and the ratio between emission and excitation wavelengths. These factors influence the basic features of the optical system. Good resolution of optical system are obtained with narrow point spread function (PSF) so as to resolve the hybridization dots located close to each other in the interphase FISH. Also, chromatic aberrations needed to be eliminated so that the individual channels are overlaid as precisely as possible. The following work has been done towards the optimization of optical microscope systems for high resolution data acquisition.

Autofocus is critical for automated microscopy, where fully automatic acquisition of microscope images in unattended operation is required. Fast and reliable autofocusing methods are indispensable for routine use of the instruments through which a large number of slides need to be scanned and analyzed. In general, autofocus algorithms determine the in-focus position for an image based on maximization of focus function, which measures the focus as a function of the axial z position and is sampled at different positions along the z-axis. The value of the focus function is computed from an image captured at that z position. Most available autofocusing techniques compute either high frequency content or intensity variance of an image as the measure of focus and they are all done at a single resolution. Multi-resolution wavelet approaches suggest a very suitable means for highly efficient autofocuing. Our results show that wavelet based approach overcomes the fundamental limitations of existing single resolution autofocusing techniques and yield superior performance [11].

Registration The calibration of the optical system to correct chromatic aberration is a typical problem for FISH imaging and microarray imaging where images are collected in more than one channel. Due to the differences in the optical paths of color channels in acquisition of FISH images and the inherent "chromatic aberration" of the optical system, these multi-spectral images do not align well with each other. This will result in inaccuracy in subsequent detection and classification of chromosomal rearrangements. This problem becomes more severe with M-FISH imaging. The optical principle underlying the inherent mis-registration problem is that the focal length of the lens changes with wavelength. As a consequence, when multiple color filters are used in M-FISH imaging, there is an offset between focal planes of different wavelengths, resulting in axial chromatic aberration. Moreover, the magnification is inversely proportional of the focal length. Hence, the same object in the specimen also changes its off-axis position when imaged with different wavelengths, resulting in lateral chromatic aberration. Similar problems also exist in a typical microarray experiment based on the hybridization using two differently labeled samples, i.e., a healthy tissue RNA labeled with Cy3 and a diseased tissue RNA labeled with Cy5. These two fluorescent images are overlaid for visual inspection and pixel-to-pixel ratio quantification. It is necessary that image alignment be performed to guarantee correct data quantification. The tedious and time-consuming step of correcting the misalignment of the different color channel images is a key factor limiting fast and cost-effective automated multicolor image analysis. There are many approaches for solving this problem. We have developed a wavelet based multi-resolution approach to tackle this problem, which has many advantages [15]. It can be used for both speeding up the search of parameters as well as for increasing the robustness of the choice of initial values.

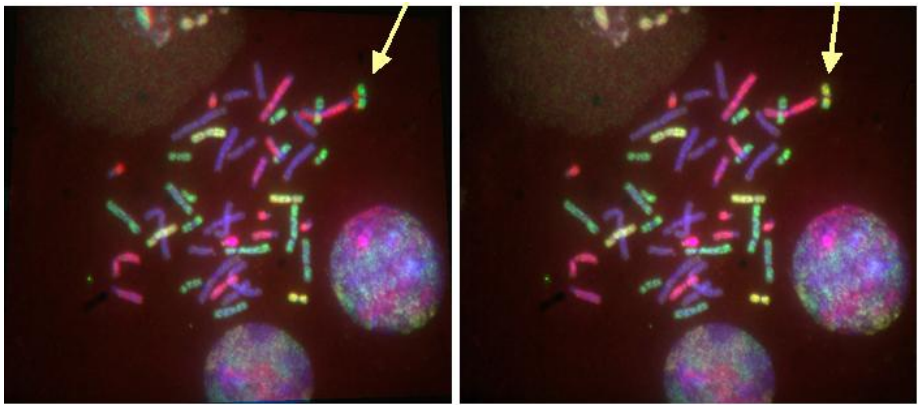

Fig.2. Effect of registration on multi-channel images. Three images from channel S.Aqua, Far Red and DAPI are combined and displayed using R,G,B colors. Before registration there is misalignment (left, indicated by arrow). After registration, good co-localization shows up (right). This misalignment, without correction, makes subsequent pixel classification less accurate.

3.2 Enhancement of chromosome images using wavelets

In FISH imaging the fluorescent specimens are often thicker than the focal depth of the microscope optics, which leads to the loss in resolution for structures that lie outside the focal plane. Deconvolution is a procedure of producing a sharper image from a blurred one. There are many approaches including 2-D and 3-D available for

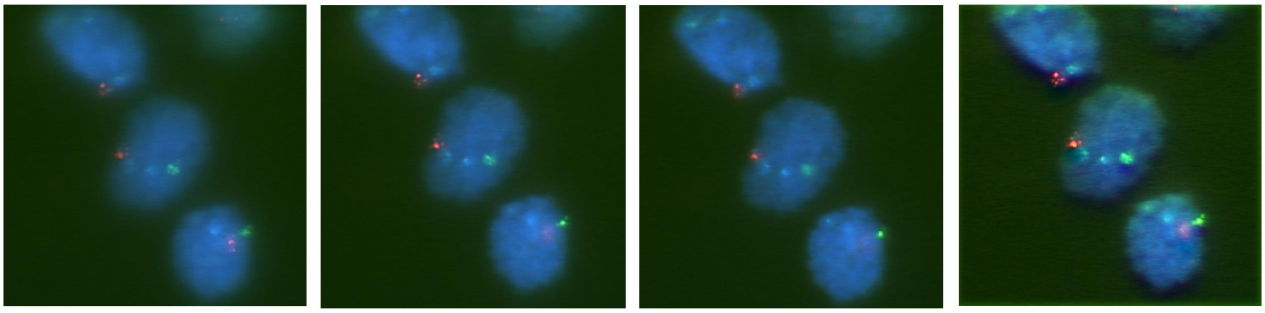

Fig3. *The deblurring of the interphase FISH images captured at three different focal planes (left three) to make the image sharper (right most) to facilitate chromosomal dot counting.*

reconstructing the image to improve the resolution of images degraded by known optical distortions. It is possible to estimate the distortion of a fluorescence microscope by measuring the point spread function. Blind iterative deconvolution approaches have also been used for this purpose. As more than one optical section image is usually available, the adjacent focal plane images can be used to remove the out-of-focus information. By taking the 3-D image slice information, the deblurred optical sections can be fused together to form a single composite image where all features are in sharp focus. A wavelet-based approach has been designed to improve the resolution of FISH-labeled cells, yielding superior performance. Fig. 3 shows such an example [14].

In G-banded karyotyping, vague band patterns of chromosomes are commonly met, due to cell culture, the limitation of staining, the presence of noise and other sources of distortion resulting from poor sample preparation, imaging and digital quantization. The enhancement of these bands would be desirable before the local band patterns are extracted. It also has a big impact on the subsequent chromosome karyotyping. We have designed a family of differential wavelets that best capture the banding patterns to enhance the useful patterns in a chromosome image while suppressing the noise. Chromosome images with and without enhancement are compared in Fig.4, showing clear banding details after the enhancement approach. Similar techniques can also be applied to FISH and microarray imaging. In order to evaluate the performance of enhancement on the karyotyping, we have done the experiment on a real world database. The images were collected from a data archive at Dyna-Gene Cytogenetics Laboratories, Houston, TX and are fairly representative of routine sample quality. The dataset has 342 G-banded cells containing15136 chromosomes [9]. The results show that images enhanced by the proposed method can result in the lowest classification error rate. These results indicate that enhanced images with the proposed method appear to provide more useful information and yield higher accuracy of classification. Hence, it will likely benefit cytogenetic diagnosis because chromosome abnormalities tend to be detected more effectively when the karyotyping accuracy improves. The enhanced images result in the increase of the downstream classification accuracy, translating into the improvement of diagnosis [9].

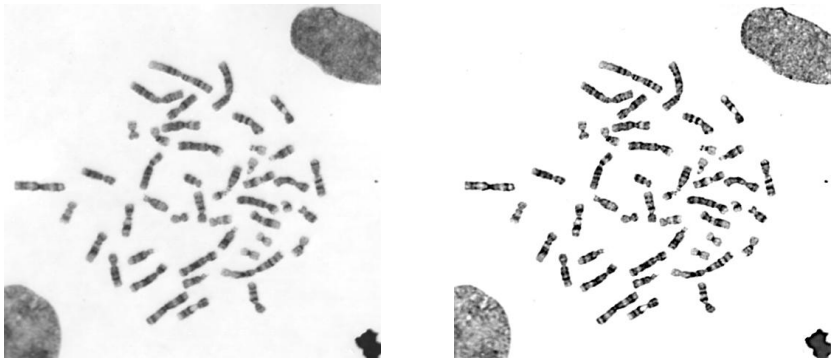

Fig4. Enhancement of G-stained metaphase chromosome images.

3.3 Chromosome image classification using wavelets features

A central problem in chromosome analyisis is automatic karyotyping, i.e., classifying human chromosomes into 24 classes according to their morphology. It is always desirable that annotated karyograms are produced with high accuracy and without the intervention of an operator. Diagnostic information can be derived from this karyogram by examining the chromosome band patterns and the complement, and relating the abnormalities to biological and clinical effects. The most robust and reliable approach is the classical maximum-likelihood Bayes classifier. Despite great effort in developing chromosome classification techniques in the last 30 years, fully automated karyotyping remains unattainable, even for normals. Most commercial systems achieve 70-80% correct classification rate in routine practice, rather than the supposed 90% accuracy the literature would suggest.

The existing approaches all assume that the banding profile of a chromosome is represented by a family of linear bases, such as the Weighted Density Distribution (WDD) functions, Principal Component Analysis (PCA), Linear Discriminate Analysis (LDA) and wavelet bases [20]. However, these linear models may not be realistic; the multiscale structure of the banding pattern can be well represented by these bases. In addition, due to the variation of cell culture conditions, chromosome staining, and microscope illumination, there are many cases in which the size and banding patterns of a specific chromosome of one cell are different from those of another cell. The multiscale bases seem more appropriate to represent the multiscale banding pattern and to reduce the variations. We have projected the banding patterns of the G-banded chromosomes into wavelets bases. The features extracted from wavelet bases are input to a Bayesian classifier. A comparison of wavelet based features with other classical bases is given in Table 1.

Classification method	WDD	PCA	LDA	Wavelet (D4)	Wavelet (D6)
Classification accuracy	87.90%	88.20%	86.50%	79.90%	80.00%

Table 1. Summary of chromosome classification experimental results on the Genzyme data set using the linear basis approaches. D4 and D6 denote the Daubechies wavelet filters of size 8 and 12.

3.4 Chromosome abnormality detection using M-FISH approach

Multiplex or multi-color fluorescence in situ hybridization (M-FISH) imaging is a recently developed cytogenetic technique for cancer diagnosis and research on genetic disorders. By simultaneously viewing the multiple-labeled specimens in different color channels, M-FISH facilitates the detection of subtle chromosomal aberrations. This technique largely depends on the accurate pixel classification (color karyotyping). The improvements in classifier performance will allow the elucidation of both more complex and more subtle chromosome rearrangement. The classification errors are caused by several sources. Misalignment or misregistration between multiple channels is a primary factor, which seriously affects the accuracy of classification. Automated registration and image normalization have to be done before the pixel classification. We have proposed a number of classifiers for multispectral pixel classification [16], [17]. In order to correct the misalignment among the different fluor images caused by color aberration and other sources of errors, an automated registration technique is introduced. The proposed registration algorithm is based on wavelets and spline approximations, having both computational advantage and improved accuracy. The effects of the registration and other preprocessing steps such as the background flattening on subsequent classification were evaluated on a comprehensive M-FISH database established by ADIR [16]. Fig.5 shows one example of the classification results. The database has collected six channel image sets at different imaging planes with probe sets from Applied Spectral Imaging (ASI), ADIR, Cytocell, and Vysis. It contains 200 spreads from 33 slides from five different laboratories. The specimens include 74 normal male, 8 normal female, 99 abnormal spreads, and 17 more that are difficult to karyotype. The evaluation indicates that wavelets based approach can allow smaller rearrangements to be identified and better enable the technique to resolve the complex rearrangements, translating into improved accuracy in identifying subtle DNA rearrangements in cancer research.

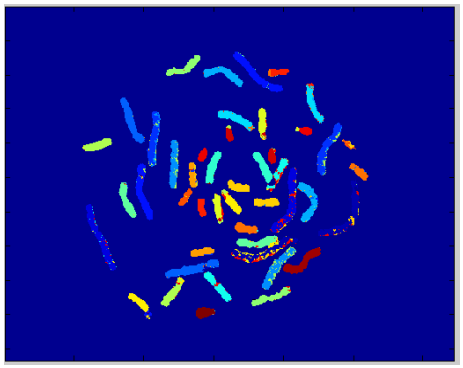

Fig.5. The real pixel-by-pixel classification of chromosomes with a Bayesian algorithm. The classification error is caused by several factors such as the misregistration.

3.5 Automated and high resolution accquision of chromosome signals

A crucial step in capturing FISH signals is to identify metaphase spreads (as shown in Figure 6), which are different from nuclei and cellular debris. Metaphase spreads are associated with higher variations in image intensity. The selection of interpretable images is usually performed by a cytogeneticist, which is labor intensive and subject to human bias. A computer vision algorithm can be used to rank the images. Metaphase images with complete sets of non-overlapped and non-overspread images are classified as nice. The other images are ranked as overlapping and overspread classes. Mathematical morphology is applied to characterize geometrical information of chromosomes, such as the length of chromosomes [16]. We have developed a wavelet based algorithm to characterize the metaphase chromosomes as fractal surface because they have different level of roughness from nuclei and debris. The algorithm can recognize the metaphase chromosomes using only these texture features [16].

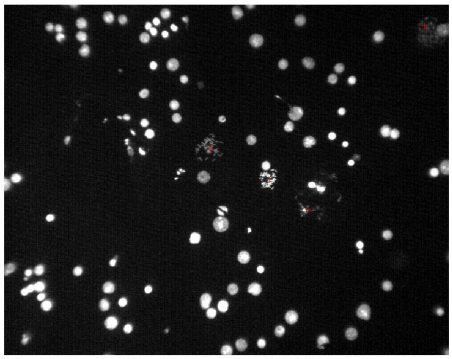

Fig.6. Detection of metaphase spreads from cells (drawn in red cross) using texture feature.

3.6 The storage and transfer of cytogenetic images

Given the vast volume of image data produced daily in a cytogenetic laboratory, compression of these data is necessary for saving of storage and reducing memory requirements. G-banding chromosome spread images typically are 764 by 560 8-bit deep if acquired by CCIR cameras. Without compression they require 427,840 bytes in storage space. After compression by GIF and PNG, the typical resulting image sizes are 222,254 and 210,158 bytes respectively. The amount of data for microarray image (usually coded with 16bit) is usually much bigger. Even one channel CGH array image can be greater than 20MB per scan. With the exponentially growing number of microarray experiments to be performed, the need of image data compression appears more pressing. Despite the cheaper storage media, there is always a need for the digital storage, archiving, and communication of cytogenetics images. A high ratio compression can be achieved encoding in the region of interest. In metaphase spread images the area containing chromosomes are well determined and segmented, and are of clinical significance. By taking advantage of this fact, a wavelet-based arbitrary region of support (AROS) coding approach was developed, which outperforms existing compression methods [10].

4. DISCUSSIONS

The trend of biomedical imaging is going from organ and tissue levels to molecular or cellular levels to determine the molecular make-up of the macro features that are currently visualized using classical imaging techniques. The cancer imaging at genome or molecular level makes it possible to detect tumors much earlier when they are easier to treat and by permitting more precise therapy or surgery. As an emerging modality, genetic imaging technique can find widespread use in the routine day-to-day portfolio of many clinical and research cytogenetic laboratories. A number of clinical specimens and cell lines have to be screened, requiring high-throughput procedure. Furthermore, it is always desirable that the automation of these genetic methodologies can be highly reliable in a speedy way. It is imperative to develop state-of- the- art image processing techniques to realize the fast and full automation of these procedures.

The potential of genetic imaging can be realized with the help of wavelet approaches. The use of wavelets has improved the reliability of these genetic screening techniques, translating to improved detection and diagnosis of cancers and genetic diseases. Without doubt, wavelet techniques will help to accelerate genetics research. Conversely, the revolutionary changes experienced now in molecular biology and genetics will give rise to many interesting but difficult problems to be solved by wavelets.

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