



Automated Prototype Generation for Multi-Color Karyotyping

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Introduction

Multi-color karyotyping is a 24-color method, first used by David Ward *et al.* [3], that allows simultaneous screening of the genome. Although, this allows visual detection of gross anomalies, misclassified pixels make manual examination difficult. Also, in the absence of prior knowledge of the anomaly, interpretation of the karyotypes can be ambiguous. We hypothesize that, generation of a single prototype from multiple karyotypes (k) for a given specimen, can highlight all of the aberrations, while minimizing misclassified pixels arising from inconsistencies in sample preparation, hybridization, and imaging procedures.

Methods and Materials

- Acquire multiple karyotypes, at least 3, from a single specimen using fluorescent in-situ hybridization as advised in the Spectra Vysion™ probe kit (Abbot Laboratories, Abbot Park, Illinois), and MFISH software™ (Applied Imaging Corp., Santa Clara, CA) [1].
- Images were acquired using a Zeiss Axioskop microscope (Zeiss, Germany) with a 6-position filter slider bar completely fitted with appropriate filter sets. A 150W mercury-xenon lamp was used for fluorescence illumination, and a Photometric SenSys™ cooled monochrome CCD camera was used to image the chromosomes. The PowerGene M-FISH digital image analysis software for MFISH was used for image acquisition, pixel classification, and color karyotyping [1].
- Prepare karyotypes for prototyping by removing all added text/graphics in the karyotype, leaving only the chromosomes and a black background.
- Analyze karyotypes with prototyping algorithm.

Approach

In this study our approach was to utilize a MATLAB (2007a, TheMathWorks™, Natick, MA) algorithm that...

- First applies Bayesian classification techniques to group homologous chromosomes across multiple karyotypes into two groups based on the similarity of color and position of the constitutive pixels in each chromosome.
- Computes the prototype chromosome by pixel-based fusion by
 - First generating an ordered set of pixels for every position (x,y) across all chromosomes.
 - Then computing the mode of the ordered set.
 - For fusion we use a simple selection criterion based on a threshold that provides a lower bound frequency of observing a specific color.
 - If the pixel color value varies from the mode, the pixel is assigned a value of zero, which means the color is uncertain and results in a black pixel in the final prototype.
- This approach allows us to generate a single prototype karyotype that reflects all anomalies for a given specimen, while rejecting non-physiological inconsistencies.

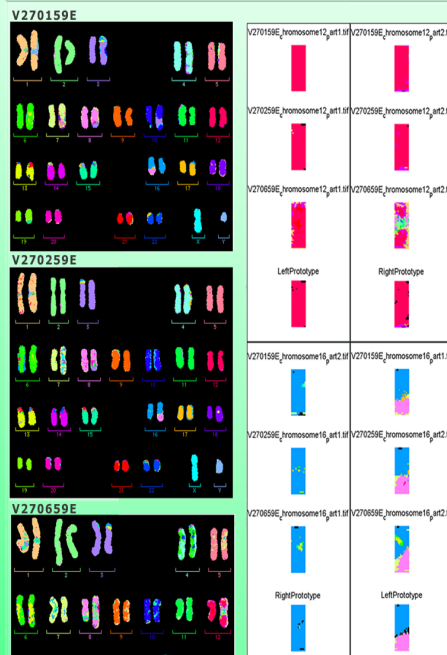


Figure 2. Sample image containing chromosomes 12 and 16 from all 3 karyotypes and the resulting prototype.

Figure 1. All 3 karyotypes from case V27_59



Figure 3. Image depicting the final prototype generated from all 3 karyotypes from figure 1.

Discussion

MFISH was used to classify pixels and create the karyotypes, and although MFISH cannot be used to identify very small insertions or translocations, it is still a very powerful technique for the analysis of complex rearrangements and the identification of chromosomal abnormalities [2]. As one can see from the above figures, the prototyping code works as described and is able to differentiate between misclassified pixels. It is able to create an accurate prototype of all three karyotypes. Figure 2 shows us how the code differentiates between pixels. Notice how the final prototype for both parts of chromosome 12 are almost unicolor, as most pixels that are not of the original chromosome color (red-pink, in this case) did not show up in later karyotypes, thus confirming the pixels as misclassified. Even the yellow-green band that appears V270659_part2 does not appear in the final karyotype, as shown in Figure 2. However, chromosome 16 part 2 appears to have a translocation of chromosome 8 on the bottom, as shown by the pink pixels in the final prototype.

Future Work

Future work planned for this algorithm include

- adding proper support for male karyotypes (currently some custom modifications have to be made everytime a male is ran due to the X and Y chromosomes)
- adding support for >2 parts per chromosome (for aneuploid karyotypes)
- optimize code (currently the code can take anywhere between 2 hours and 7 days to run).

References

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