Quantitative FISH Image Analysis for Telomere Length Measurements

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Quantifying the amount of a specific DNA sequence in a specimen is increasingly important in cytogenetics laboratories as genetics research reveals more detailed information about the human genome and gene expression. Cytogeneticists both in clinical practice and in cancer research are increasingly interested in quantitative information about the presence or absence of specific DNA sequences in their specimens. One area of growing interest is in quantifying the amount of telomeric DNA in cancer specimens, since telomeres are important to the stability and replication of chromosomes. Cancer cells that have long telomeres are postulated to be more likely to metastasize than cancer cells that do not. We have developed image analysis tools to perform telomere length measurements using fluorescence microscopy. These tools have the potential to improve significantly the clinical diagnosis of cancer, and will be useful for finding small chromosome deletions that presently escape detection using FISH techniques and for gene expression studies.

The brightness of a FISH spot in a microscope image is proportional to the number of fluor molecules present, there are a fixed number of fluor molecules per nucleotide in the DNA probe, and the nucleotides hybridize proportionally to the chromosomal DNA. Thus, the spot brightness is proportional to the amount of the target DNA sequence present. For telomeres, the target DNA sequence is TTAGGG. We will present data that show that specimens with different telomere lengths can be easily distinguished using quantitative FISH analysis, and furthermore that the length differences can be accurately quantified.

Our approach is to use digital image analysis methods to obtain accurate total fluorescence measurements for FISH-labeled structures. We use surface fitting and background subtraction for image flattening, grayscale linearization and normalization, and color compensation to prepare the images for computing integrated fluorescence brightness for each labeled structure of interest. An example of telomere length measurement from fluorescence microscope image analysis is given in Figure 1 below. The specimen in Figure 1(*b*) is shown to have significantly more telomeric DNA than the specimen in Figure 1(*a*).

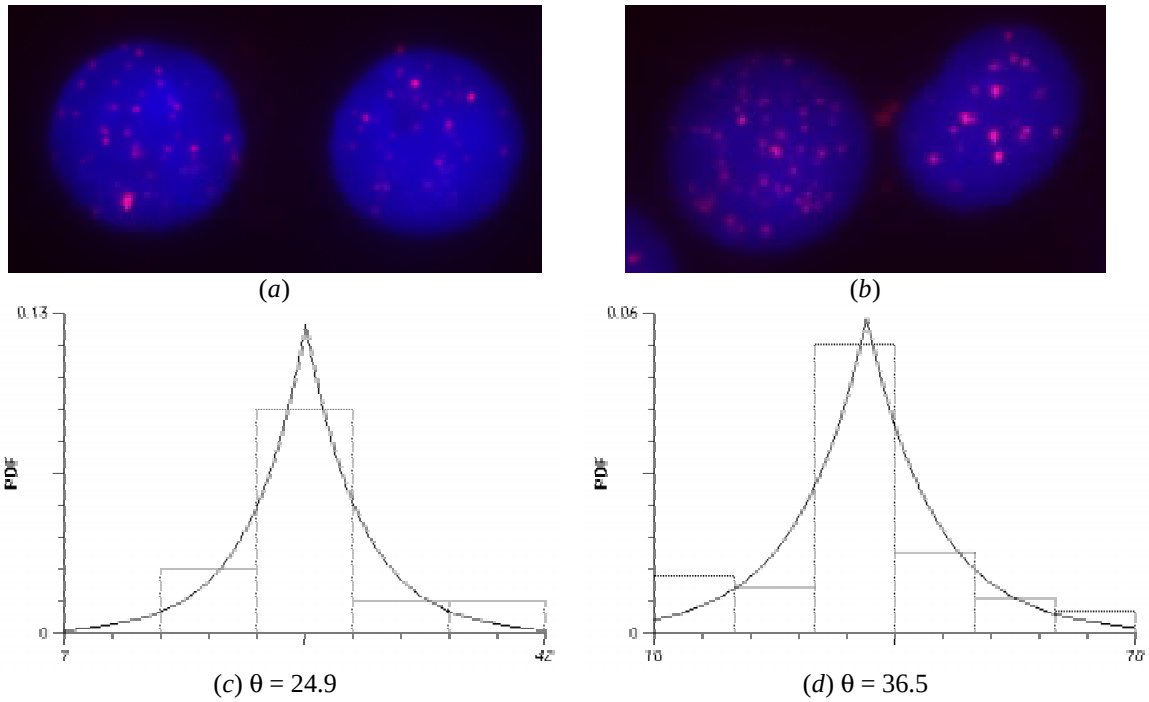

Figure 1. (*a*) and (*b*) Images of interphase nuclei of two different types of prostate cancer cells in blood culture with telomeric DNA labeled with Cy3 (red); (*c*) and (*d*) Histogram of the measurements from (*a*) and (*b*) and plot of the maximum-likelihood fitted Laplace (double-exponential) probability density function.