parameters can be used as a gate to create subpopulations with the desired properties. Parameters can be simple fluorophore intensities that measure expression of a gene or protein, e.g. in transient transfection, or utilize more complex measurements of fluorophore location and morphometrics within subcellular compartments and its organelles. Once all parameters for assay preparation, image analysis, and data mining are determined, the designed assay can be applied in a high-throughput setting using all levels of automation. To show examples of enabling assay design, image cytometric parameters have been used to identify a subpopulation (< 5% transfection efficiency) of GFP-labeled wild-type and mutant androgen receptors transiently transfected into HeLa cells. Creating a stably transfected cell line had not yet been successful and population analysis for the desired parameters was therefore not possible without weeks of FACS sorting. Using the subpopulation, dose response curves were generated based on measurements of foci formation and nuclear translocation. Similar techniques can be used in ultra-rare cell detection of fetal nucleated red blood cells and breast cancer cells in circulating blood. The tremendous efficiencies gained by HTM, data mining and virtual sorting at high resolutions and speeds create advanced cell population analyses that will enable completely new experiments in drug discovery, diagnostics, cancer screening, cytopathology and fundamental cell biology research, even when homogeneous populations cannot easily be established.

97077

ANALYSIS OF CHROMATIN TEXTURE BY PINKUS' APPROXIMATE ENTROPY

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Pincus' approximate entropy (ApE) has been originally used for the analysis of time series where it provides information on the complexity of both deterministic and random processes. It measures the probability that runs of patterns that are close to each other will remain close in the next incremental comparisons. ApE may be interpreted as the information-theoretic rate of entropy for approximating Markov chains. The aim of our study was to investigate whether ApE could be used for texture analysis of chromatin. We acquired gray-scale transformed digitalized images of cytologic preparations. The 2D images of the segmented cells were transformed into 1D signals (of about 5000 to 8000 pixels) by peel-off-scanning. This was done by applying a spiral scan algorithm beginning at the periphery of the nucleus. Then the pixel row was divided into blocks of 512, 1024 or 2048 pixels. The input variables were defined between 1 to 5 and the tolerance varied between 10 to 30 % (in steps of 5) of the standard deviation of the data sets. For each patient ApEs of these 75 combinations were calculated as the avarage values of all blocks or of the peripheral or central parts of the nuclei separately. We evaluated the relevance of ApE in two different biological models: 1. Model. Physiological maturation of the rat heart: ApEs were calculated from hematoxylin-stained cytologic preparations of nuclei of cardiomyocytes of normal rats (7 different ages between 19 days of fetal age and 60 days post partum; 100 nuclei per rat ; total of 90 rats). In all ApE combinations the Kruskall Wallis test indicated significant differences in at least one age group, thus indicating differences of the chromatin pattern. We calculated moderate to strong Spearman correlations between ApEs and the age of the animals (up to r =–0.83), as well as the mitotic count (up to r = 0,62). 2.Model. Bronchial brush cytology: ApEs of nuclear images of brush cytology of 40 patients were determined, comparing cases with and without neoplasia. Significant differences between the groups could be detected with high ApE levels for oat cell carcinomas and relatively low levels for squamous cell and adenocarcinomas. Our investigations suggest that the determination of ApEs may be useful for texture analysis of chromatin. Supported by CNPq and FAPESP.

96580

GRANULOMETRIC RESIDUES AS A DIAGNOSTIC TOOL IN CYTOLOGY

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In routine pathology, the assessment of the chromatin texture plays a important rule in the diagnosis of neoplastic differentiation. Classic morphometric parameters fail to describe patterns of distribution of heterochromatin in nuclei. Granulometry characterizes quantitatively the coarseness of an image in a hierarchical way by means of a decomposition. It obtains an ordered and complete description of an image from morphologic residues, which are defined as the difference between two consecutive granulometric levels [1]. The aim of this study was to investigate the texture of chromatin in order to evaluate the cell differentiation in cytology. From each granulometric level of gray scale images the residues were extracted by progressive filtering (openings and geodesic reconstruction, using the height of the basins (in gray levels) as filter parameter. The number of residues and their mean area was registrated for each granulometric level (between 1 to 128). Two different biological models were investigated: Model 1: We analysed cytologic preparations of hematoxylin stained nuclei of KOH-hydrolised cardiomyocytes of normal rats of 7 different ages (between 19 days of fetal age and 60 days post partum; 100 nuclei per rat; total of 90 rats). The Spearman correlation coefficients between age granulometric parameters were calculated. We found that mean areas of residues of the levels 9 to 40 described differences according to the age. With increasing age larger residues could be found. The number of residues with granulometric levels from 12 to 57 was increased in the younger ages. These results demonstrated chromatin changes during the normal development of the cardiac tissue. With increasing age, the nuclei became more elongated, while the chromatin texture was getting smoother. Sometimes tiny nucleoli appeared. Model 2: We compared nuclear images of HE-stained bronchial brush cytology of 40 patients either without neoplasia or with primary pulmonary tumors. Significant differences between the pathologic entities were found for the number of residues with granulometric level < 12 and for the mean areas with granulometric levels >9. In general neoplastic nuclei show fewer residues with larger mean area. We conclude that the extraction of granulometric features may be useful for the texture analysis of chromatin. Supported by CNPq, FAPESP.

96975

FRACTAL DIMENSIONS APPLIED TO THICK CONTOUR DETECTION AND RESIDUES—COMPARISON OF KELOIDS AND HYPERTROPHIC SCARS

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The fractal dimension (FD) has shown to be an important tool in image analysis. Recently morphological granulometric moments have proven to be very useful for the quantification of texture. The aim of our study was to combine both techniques and apply them to the study of skin biopsies of patients with keloid or hypertrophic scar.Both entities show distinct clinical behaviour and require different therapy, but may be confused on routine histopathologic examination. The aim of our study was to investigate whether the fractal dimensions of images under thick contour detection and of granulometric residues could help to distinguish both lesions. Twenty-two patients with keloids and patients with 12 hyper-