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

Konradin Metze[1], Randall Luis Adam[1], Patricia Villalobos Silva[2], Rita Barbosa De Carvalho[3], Neucimar Jerônimo Leite[4]

[1]State University of Campinas, CAMPINAS-SP, São Paulo, Brazil; [2]Catholic University Campinas, Campinas, São Paulo, Brazil; [3]State University of Campinas, Pathologuy, Campinas, São Paulo, Brazil; [4]State University of Campinas, Institute of Computing, Campinas, SP, Brazil

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

Randall Luis Adam[1], Neucimar Jerônimo Leite[2], Rita Barbosa De Carvalho[1], Patricia Villalobos Silva[3], Konradin Metze[1]

[1]State University of Campinas, Campinas, São Paulo, Brazil; [2]State University of Campinas, Institute of Computing, Campinas, São Paulo, Brazil; [3]Catholic University of Campinas, Campinas, São Paulo, Brazil

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

Randall Luis Adam[1], Tereza Cristina Gondim Corsini[1], Patricia Villalobos Silva[2], Maria Letícia Cintra[1], Neucimar Jerônimo Leite[3], Konradin Metze[1]

[1]State University of Campinas, Campinas, São Paulo, Brazil; [2]Catholic University Campinas, Campinas, São Paulo, Brazil; [3]State University of Campinas, Institute of Computing, Campinas, São Paulo, Brazil

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-

trophic scars entered the study. Diagnosis had been obtained by two observers based on standard histologic examination of paraffin embedded material as well as on clinical features. In each case 20 images of Masson-stained routine paraffine sections were adquired and gray-level transformed.A differential box-counting method in order to obtain FD from gray images was applied. FDs were obtained from the original image and from images representing 10 successive differences between the original and the dilated images, defining a multiscale fractal information. Furthermore we examined the FDs of morphological residues obtained by the difference between increasing (consecutive) morphological openings in 10 successive steps. Thus the sequence of the FD values under increasing filterings created a "profile" for each image. The mean values of the FD's derived from the granulometric residues decreased significantly with increasing filtering. The highest FDs were achived after 3 dilations. The mean FD of the original image, all the FDs after dilation, as well as the FDs of the residuals of level 2 to 8 revealed significant differences with higher FDs for the hypertrophic scar. Thus there was a difference in the absolute FDs of the "profile" between both pathological entities but not in its shape. In summary, FD of a 2D gray scale image may increase after thick contour detection. The FD of the gray level image provides useful information to differentiate keloids from hypertrophic scars, even after several dilations. This fact can indicate that FD is robust to intensive filtering of the image (e.g. out of focus images). The value of the profile created from the FD values has to be tested in other biological models. Supported FAPESP, CNPq.

96363

OPPORTUNITIES FOR APPLYING DIGITAL AND ANALOGOUS MACHINE VISION IN CELL ANALYTICAL METHODS USED FOR ANALYZING CELL DISRUPTION EFFECT OF ULTRASOUND

Attila Lõrincz[1]

[1]University of West Hungary, Institute of Agricultural, Food and Environmental Engineering, University of West Hungary, Mosonmagyaróvár, GYMS, Hungary

During the examinations *in vitro* Saccharomyces cerevisiae yeast samples were irradiated with ultrasound having an output of 9W cm-2 and a frequency of 1.117MHz. The vitality of the cells during the ultrasound treatment was examined by vital dyeing. Color and brightness of the cells was changed immediately when they were destroyed. The treated yeast sample was circulated in a flow-injection system between the ultrasound irradiation field and the optically detector. Light microscope equipped with a CCD camera was used as an optical detector. Slide of the light microscope was replaced by an optical flow-injection cell and the treated sample was circulated through this. From the image of CCD camera the survival dynamics and the decimation periods of the examined species were determined by using analogous and digital machine vision methods. For analogous machine vision, a pseudo-color coding system was built that was capable of evaluating different degree of the grey scale of CCD image. For digital imaging the Image Pro Plus 4.5 software was used. During the active ultrasound irradiation, a series of acoustic phenomena occurred in the suspended samples and each phenomenon has different intensity of cell damaging effect. This provided a good opportunity for the manual validation of the survival results determined by machine vision methods. By comparing the different examined methods, conclusions regarding their practicability and limitations in determining the number of living cells were drawn. By means of the applied machine vision systems, the quantity and quality of the individual cell types and cell organelles and for example, the environmental toxicity can be determined in real time.

Parallel Session 4: Rare Events

Chair: Carl-Hernik Brogren

96742

CELLTRACKS™ AUTOPREP AN AUTOMATED SAMPLE PROCESSOR FOR IMMUNO-MAGNETIC ENRICHMENT AND FLUORESCENT LABELING OF CIRCULATING TUMOR CELLS (CTC)

Michael Kagan[1], Theresa Bendele[1], Jared Mayes[1], Robin Levy[1], Doug Keen[1], Douglas Paynter[1], Chandra Rao[1], Melissa Hermann[1], Grace Martin[1], Leon W.M.M. Terstappen[1]

[1]Immunicon Corporation, Huntingdon Valley, Pennsylvania

Introduction: The presence and number of CTC in patients with metastatic carcinomas is associated with shorter progression free survival and survival. To accurately and consistently enumerate CTC, automated processing of blood samples before analysis of these rare cells is required. **Method:** The CellTracks™ AutoPrep was developed to automate the isolation of CTC in 7.5 ml blood aliquots drawn in CellSave™ blood collection tubes. Enrichment of CTC is accomplished by adding immuno-magnetic particles to the sample and applying magnetic fields during both incubation and separation. Several separation steps are performed sequentially, with decreasing amounts of fluid added to resuspend the collected cells. Fluorescent labeling of CTC is accomplished by addition of staining reagents during the enrichment steps, excess reagents are removed by magnetic washes and free colloidal magnetic particles are reduced by aspiration. The resulting sample is contained in a volume of 320 ul and placed in a CellSpotter® or CellTracks™ cartridge. The cartridge is contained within a magnetic holder that moves the cells to the optical transparent upper surface of the cartridge. The sample can be presented to the Immunicon CellSpotter® or CellTracks™ Analyzer for analysis. Studies were conducted to assess the reproducibility of the system. **Results:** The system processes up to eight whole blood samples in 3.5 hours with no operator interaction once the batch process has started. The first sample is ready 2 hours after the batch has started, with remaining samples available every 12 minutes thereafter. Reagents for sample processing are housed in a self-contained carrier that interfaces with the instrument, requiring no operator intervention. 72 samples were spiked with approximately 1000 and 50 cultured tumor cells and were processed on 3 different instruments by 3 different operators, over several days. Recovery of cells spiked at the 1000-cell level ranged from 752–1095 (mean 986 + 56, cv= 5.7%) and at the 50-cell level the range was 27-62 (mean 47 + 7.0, cv=14.9%). Comparison of blood samples from carcinoma patients processed manually and with the automated system demonstrated that CTC present in these samples were preserved throughout the sample processing. **Conclusion:** CellTracks™ AutoPrep is a fully automated system that can consistently immuno-magnetically enrich and fluorescently label CTC from 7.5 ml blood volumes. Additional rare cell applications are planned for development. The samples can be analyzed by Immunicon CellSpotter® or CellTracks™ Analyzer or other cell analysis platforms such as flow cytometry.