

## IMAGE ASSISTED CYTOMETRY: ANALYTICAL CAPABILITIES OF LASER SCANNING CYTOMETRY

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By offering high throughput rates, sensitivity and possibility for multiparameter analysis flow cytometry (FC) contributed immensely to the progress in various disciplines of biology, medicine, and biotechnology. For many applications, however, the analytical capabilities of FC are inadequate. The microscope-based laser scanning cytometry (LSC) provides the means to interrogate individual cells with rates and sensitivity comparable to FC but offers a plethora of analytical capabilities that FC does not have. The multi-laser fluorescence excitation and four channel emission readout allows one to concurrently use different fluorochromes for multi-spectral analysis by LSC. Unlike most fluorescence image analysis (FIA) instruments intensity of fluorescence in LSC is measured by photomultipliers providing thus higher dynamic range of fluorescence intensity measurement. The following capabilities of LSC were found to be useful in many applications: (a) position of each cell on the slide is recorded which makes it possible to sequentially analyze the same cells using different immuno- or cytochemical stains or genetic probes. The “merge” capability can be then used to integrate the data of the sequential measurements thereby providing information on cell function, immunophenotype, expression of particular protein, DNA ploidy, cell cycle phase, and/or cytogenetic profile. A combination of the cell attributes measured supravivally with the attributes measured post-fixation can be integrated for each measured cell; (b) using the merge function cell populations may be examined repeatedly over time e.g. to study enzyme kinetics, drug uptake rate and other time-resolved processes; (c) any of the measured cells can be relocated for its fluorescence or bright-field microscopy image analysis; (d) subcellular topographic distribution of fluorescence intensities can be measured for analysis of translocation of cell signaling molecules or transcription factors (e.g. NF- $\kappa$ B, p53) between cytoplasm, nucleus and other cell organelles; (e) local hyper- or hypo- chromicity of the fluorochrome reported by the maximal pixel intensity of fluorescence allows one to measure degree of chromatin condensation, a feature used to identify mitotic or apoptotic cells, discriminate between lymphocytes, granulocytes and monocytes, and detect the early stage of DNA damage response, namely chromatin relaxation. It also allows the detection of such events as receptors clustering or a change in degree of local density of the molecules e.g. reflecting translocation of Bax to-, or cytochrome c from- mitochondria, as the event initiating apoptosis; (f) the capability of quantification of individual fluorescent foci per cell or nucleus makes it possible to use it for the semi- automatic analysis of the fluorescence *in situ* hybridization (FISH), for micronucleus assay in mutagenicity tests, or for enumeration of immunofluorescent foci of the DNA damage-associated molecules (e.g.  $\gamma$ H2AX, ATM), the markers of the DNA double strand breaks. The multivariate analysis of the above parameters reveals a wealth of information on association between extent of DNA damage, recruitment of DNA repair machinery, activation of cell cycle checkpoints and induction of apoptosis. The image-assisted cytometry as exemplified by LSC instrumentation bridges the gap between the zero-resolution cytometry such as FC and high-resolution image analysis, thereby complementing the range of analytical capabilities available for the cell biologist.