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Towards *in vivo* flow cytometry

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Cytometry is the characterization and measurement of cells and cellular constituents for biological, diagnostic and therapeutic purposes, embracing the fields of cell and molecular biology, biochemistry, biophysics, cell physiology, pathology, immunology, genetics, biotechnology, plant biology and microbiology. Cytometry is based on quantitative measurements of the molecular and phenotypic properties of cells using flow and image cytometry, microarrays, and proteomics.

Cytomics (molecular cell systems research) aims at the understanding of the molecular architecture and functionality of cell systems (cytomes) by single-cell analysis in combination with exhaustive bioinformatic knowledge extraction. The cytomics concept has been significantly advanced by a multitude of current developments like confocal and laser scanning microscopy, multiphoton fluorescence excitation, spectral imaging, fluorescence resonance energy transfer (FRET), fast imaging in flow, optical stretching in flow, and miniaturized flow and image cytometry within laboratories on a chip or laser microdissection, as well as the use of bead arrays [1, 2].

Data sieving or data mining of the vast amounts of collected multiparameter data for exhaustive multilevel bioinformatic knowledge extraction avoids the inadvertent loss of information from unknown molecular relations being inaccessible to an a priori hypothesis. These approaches will become powerful tools for such important fields as individualized medicine, drug discovery and drug development [3–6].

This special issue is focused on state-of-the-art research in advanced cytometry and application areas with particular emphasis on novel biophotonic methods, disease diagnosis, and monitoring of disease treatment at single-cell level in stationary and flow conditions. It seeks to advance scholarly research that spans from fundamental interactions between light, cells, vascular tissue, and labeling particles, to strategies and opportunities for preclinical and clinical research.

Recent advances in slide-based cytometry for *in vitro* application are summarized by Gerstner et al. [7]. They show that single cell based quantitation using microscope based cytometry instruments is making its way from basic research into clinical use. Calibration and quality control are essential in every cytometric analysis. Basics of standardization and

calibration in cytometry are comprehensively summarized by Mittag and colleague [8]. Leif [9] demonstrates how data formats produced by cytometric measurements can be integrated into clinical diagnostic system standards for improved data management in the clinical environment.

Flow cytometry (FCM) is based both on cell detection as whole and the use of high-speed imaging of individual cells and nanoparticles. Tychinski and co-workers [10] analyze the metabolic component of cellular refractivity and its importance for optical cytometry. The refractivity decrease in response to energy depletion was demonstrated for mitochondria, chloroplasts, spores, and cancer cells using the coherent phase microscope “Airyscan”. Estimations indicated high values for the equivalent refractive index of structured water in cells.

To reduce the interference from tissue light scattering background, gold nanoparticles with strong plasmon scattering resonance properties can be applied as *in vivo* FCM contrast agents. This technique requires an understanding of light interaction features of cells with and without nanoparticles. Tanev and coworkers [11] compare light scattering of these cells in controlled refractive index matching conditions. They analyze the optical schematics including phase contrast microscopy as a prospective modality for *in vivo* FCM.

Jacobs et al. [12] consider some important features of the diffraction imaging of spheres and melanoma cells with a microscope objective. Using a confocal imaging-based method to reconstruct and analyze the 3D structure, they demonstrated that genetic modifications in these cells can induce morphological changes, and the modified cells can be used as an experimental model to study the correlation between 3D morphology features and diffraction image data.

Galanzha et al. [13, 14] introduce *in vivo* FCM with photoacoustic and photothermal detection schematics. Conventional FCM provides fast multi-parameter quantification of the biological properties of individual cells at subcellular and molecular levels. Nevertheless, the invasive extraction of cells from a living system modifies their properties, preventing their long-term study in their native environment. *In vivo* FCM of blood and lymph vessels with natural bioflows overcomes these problems. Cells can be detected label-free, by endogenous chromophores and pigments or by advanced strongly absorbing nanoparticles as novel labels. Both approaches are demonstrated for noninvasive rapid detection and treatment of metastases in lymph flow and sentinel lymph nodes by an integrated system of multicolor photoacoustic lymph FCM/lymphography and photothermal therapy.

Bykov et al. [15] employ Doppler OCT imaging of cytoplasm shuttle flow in a slime mold *Physarum polycephalum*. Radial contractions of the gel-like walls of the strands and the velocity distributions in the sol-like endoplasm streaming along the plasmodial strands are imaged. The motility inhibitor effect of carbon dioxide on the cytoplasm shuttle flow and strand wall contraction is shown.

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Biographies

Valery V. Tuchin holds the Optics and Biophotonics Chair and is a Director of Research-Educational Institute of Optics and Biophotonics at Saratov State University. He has authored more than 300 peer-reviewed papers and books, including his latest, Tissue Optics (PM166, SPIE Press, 2007) and Handbook of Optical Sensing of Glucose in Biological Fluids and Tissues (CRC Press, 2009). He has awarded RF Honored Science Worker and SPIE Fellow; he is a Vice-President of Russian Photobiology Society. In 2007 he has been awarded by SPIE Educator Award.

Attila Tárnok obtained his Ph.D. in 1988 from the University of Hamburg, Germany at the Institute for Biophysics and Radiation Biology. He habilitated in 2000 and since 2006 has been Professor for Immunology and Cytomics at the University of Leipzig, Germany. Most of his work has focused on development for cytometry and cytomics for slide-based and flow systems on microanalytical multiplex assays for humoral and cellular immune diagnostic. He is Editor-in-Chief for Cytometry Part A, published more than 200 peer-reviewed articles and book chapters, guest edited several special issues of scientific journals and is the editor of textbooks.

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