Cell Prolif. 2006, **39**, 495–505

Cytomics – importance of multimodal analysis of cell function and proliferation in oncology

A. Tárnok*, J. Bocsi* and G. Brockhoff†

**Department of Paediatric Cardiology, Cardiac Centre Leipzig GmbH, University of Leipzig, Leipzig, Germany,*
†Institute of Pathology, University of Regensburg, Regensburg, Germany

Received 30 *August* 2006; *revision accepted* 9 *September* 2006

Abstract. Cancer is a highly complex and heterogeneous disease involving a succession of genetic changes (frequently caused or accompanied by exogenous trauma), and resulting in a molecular phenotype that in turn results in a malignant specification. The development of malignancy has been described as a multistep process involving self-sufficiency in growth signals, insensitivity to antigrowth signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and finally tissue invasion and metastasis. The quantitative analysis of networking molecules within the cells might be applied to understand native-state tissue signalling biology, complex drug actions and dysfunctional signalling in transformed cells, that is, in cancer cells. High-content and high-throughput single-cell analysis can lead to systems biology and cytomics. The application of cytomics in cancer research and diagnostics is very broad, ranging from the better understanding of the tumour cell biology to the identification of residual tumour cells after treatment, to drug discovery. The ultimate goal is to pinpoint in detail these processes on the molecular, cellular and tissue level. A comprehensive knowledge of these will require tissue analysis, which is multiplex and functional; thus, vast amounts of data are being collected from current genomic and proteomic platforms for integration and interpretation as well as for new varieties of updated cytomics technology. This overview will briefly highlight the most important aspects of this continuously developing field.

INTRODUCTION

Cytomics is bioinformatic knowledge extraction, from a large amount of structural and functional information by molecular cell phenotype analysis of tissues, organs and organisms, at the single cell level by image or flow cytometry (Valet 2005a,b). Single-cell analysis by cytometric methods has equally reached high-throughput capacity in recent years, permitting the assessment and quantification of the molecular morphology of single cells (Tarnok 2004, 2006; Valet *et al*. 2004). Typical investigations use hypothesis-driven parameter selection in combination with hypothesis-free exhaustive knowledge extraction, that is, cytomics (Kitano 2002; Hood

Correspondence: Prof Dr Attila Tárnok, PhD, Department of Paediatric Cardiology, Cardiac Centre, University of Leipzig, Strümpellstr. 39, D-04289 Leipzig, Germany. Tel.: +49-341-8652430; Fax: +49-341-8651143; E-mail: tarnok@medizin.uni-leipzig.de

et al. 2004; Kriete 2005; Murphy 2005; Valet 2005b). Although a given hypothesis can be proved or disproved by a given experiment, the evaluation of all cell data in a hypothesis-free fashion (discovery science) enables the exploration of unknown multiparametric data spaces. The secondary results thus generated are subsequently available for new hypothesis development. This approach reflects the reality that cells and their inter-relationship and not genes or biomolecules represent the elementary functional units of organisms. Diseases are caused by molecular alterations in cells or cellular systems (cytomes) as a consequence of genotype and exposure to external or internal influences.

Cytomics in oncology

It is evident that systemic cell analysis has instant impact on oncology and cancer research (Celis *et al*. 2005; Khalil & Hill 2005; Abramovitz & Leyland-Jones 2006; Hornberg *et al*. 2006; Liu *et al*. 2006). It helps unravel molecular circuits underlying cell proliferation, cancer development and ultimately, treatment (Alberghina *et al*. 2004; Spencer *et al*. 2004; Tabach *et al*. 2005; van Leeuwen *et al*. 2006).

Flow cytometry (FCM) is a very powerful tool for metabolic and dynamic analysis of living single cells. It is the method of choice for understanding how a system behaves over time and under various conditions (Sachs *et al*. 2005). Crucial molecular mechanisms underlying specific kinds of cellular behaviour for example, differentiation, cell death, growth and cell proliferation (Yarden & Sliwkowski 2001), can be identified using FCM. Quantitative analysis of networking molecules within cells might be applied to understand native-state tissue signalling biology, complex drug actions and dysfunctional signalling in diseased cells such as cancer cells. Awareness is evolving that intra-cellular signal diversity and specificity, initially triggered by extracellular signals, is based on highly complex networking, inter-pathway cross-talk, rather than by individual pathways or model systems in isolation (Yarden & Sliwkowski 2001; Jones *et al*. 2006). Multivariate analytical and single-cell–based cytometric approaches are required to understand normal cellular physiology and the potential dysregulation that might lead to malignant disease (Chassagnole *et al*. 2006). Complex and highly dynamic analysis of intra-cellular signalling pathways can be performed flow cytometrically by quantifying the activation of pathway components under unaffected and under perturbed conditions using, for example, specific kinase inhibitors disturbing a functional system at different levels. The complementary application of (intra-cellular) multicolour, high-content FCM (Edwards *et al*. 2004) and Bayesian network analysis allows the description of direct and indirect causal interactions of pathway components and therefore the generation of contextual images of networks within the cell, derived from quantitative analysis of thousands of individual cells (Sachs *et al*. 2005).

Multiparameter FCM is a particularly appropriate source of reliable quantitative data that enables, in combination with computational network analysis, to decipher the importance and regulative impact of pathway components on each other (Valet 2003; Wiley *et al*. 2003). High-content, quantitative analysis and network construction can be performed without *a priori* knowledge of pathway connectivity, which would warrant circumvention of loss of valuable and essential information (Perfetto *et al*. 2004; Bernas *et al*. 2006). Appreciation of both biological diversity of cancer and tumour cell heterogeneity is forcing treatment into patterns that reflect the underlying cellular features of the tumour. Principles of therapy may need to be redefined for each distinctive class of cancer, based on comprehensive analysis of molecular systems and vital cells.

Functional proteomics has been applied to comprehensively investigate the capacity of highly related erbB-receptor tyrosine kinases to interact with intra-cellular pathway molecules (Uetz & Finley 2005; Abramovitz & Leyland-Jones 2006; Uetz & Stagljar 2006). erbB receptors

are of particular importance in pathological diagnosis of numerous epithelial malignancies (Lottner *et al*. 2005). Abnormal expression and/or activation of this gene/protein has a severe negative impact on the course of disease in breast, ovarian, head and neck, colon and lung cancers on the one hand, and this is of importance for therapeutic strategy on the other (Richter & Zhang 2005). However, capability of the four erbB-receptor members (epidermal growth factor receptor, Her2–4) to interact laterally with each other and to trigger intra-cellular signalling collectively has been incompletely understood and needs a comprehensive investigative approach to be deciphered (Yarden & Sliwkowski 2001). Intra-cellular phosphotyrosine binding sites have been the subject of unbiased investigation concerning their potential to interact with intra-cellular signalling molecules and to trigger specific pathways (Schulze *et al*. 2005; Jones *et al*. 2006), which result in malignant cell growth and proliferation. Shared and unique features of individual erbB molecules have been identified to be responsible for their specific promiscuity, a crucial feature of their malignant potential. However, the impact of specific and individual co-expression profile of these receptors on lateral interaction and intra-cellular signalling can be expediently investigated in living cells (Brockhoff *et al*. 2001; Diermeier *et al*. 2005). Fluorescence resonance energy transfer (FRET) is a highly sensitive and quantitative technique to investigate single-cell–based receptor interaction (Vereb *et al*. 2004). In combination with dynamic read-out parameters, for example cell cycle kinetics, the dysregulation of cell proliferation as a crucial marker malignant cell growth, can be accurately and sensitively analysed (Brockhoff *et al*. 2001; Diermeier *et al*. 2005). Dynamic multiparameter analysis will contribute significantly to elucidate inter-relationships of molecular components within molecular systems (for example, the erbB-receptors) and their regulation, at the cellular level of normal and diseased cells. Multiparametric FCM will contribute significantly to specific patient selection for individualized therapy. A detailed tumour cell analysis, which is available after analytical methods in cytomics, has the potential to increase the ratio of therapy responders to non-responders and will minimize cases of patient over-treatment. In addition, a cost-effective patient treatment with, for examples Trastuzumab™ (Roche Diagnostics, Penzberg, Germany), can be achieved (Elkin *et al*. 2004). In oncology, the now emerging multiparametric approaches to uncover cells' protein networks have the potential to determine optimal treatment improving both survival and quality of life for an individual patient (Valet & Hoeffkes 2004). Combining dynamic and functional FCM entails the capacity of establishing predictive medicine in order to identify patients at risk of disease manifestation or of developing complications and side effects to a certain treatment (Tarnok *et al*. 2006). Utilizing dynamic and functional FCM for novel and highly specific therapeutic targets expected to help identify and optimize patient stratification based on quantitative cell and tissue analysis (Ecker & Steiner 2004; Lottner *et al*. 2005; Mosch *et al*. 2006), will follow.

Innovative technologies for systemic cell analysis

Cytomic studies on cascades of information that flow through signalling pathways need complex analysis of individual pathway components. Knowledge of carcinogenesis and tumour progression, and the variety of intra-cellular physiological processes triggered by anticancer treatments, are continuously extending. Polychromatic and functional FCM as well as slide-based cytometry (SBC) are key technologies to achieve this goal. Clearly, light microscopic analysis of cells and tissues, in conjunction with imaging – sophisticated image analysis, and data display – are central for automated high-content single-cell–based analysis. SBC analysis can combine both single-cell genomics and proteomics, with structural analysis of cells in their natural environment (for example in whole tissues and/or in cell cultures) (Tarnok & Gerstner 2002; Ecker & Steiner 2004; Gerstner *et al*. 2004a; Megyeri *et al*. 2005). Quantitative cytometric analysis of tissue sections can only be performed with the aid of SBC technology. Thus, a multitude of single-cell features

can be captured simultaneously with high-throughput single-cell microscopy (Steiner *et al*. 2000; Schubert 2003, 2006; Bocsi *et al*. 2004; Ecker *et al*. 2004; Gerstner *et al*. 2004a; Kantor *et al*. 2004; Perlman *et al*. 2004; Varga *et al*. 2004; Mittag *et al*. 2005, 2006) and can be reconstituted to single-cell 3D tissue architecture (called tissomics), to provide data-rich profiles of cellular heterogeneity in tissues (Ecker & Tarnok 2005; Kriete & Boyce 2005; Kriete *et al*. 2006; Mosch *et al*. 2006). Phenotypical data are covariants that can be used as biomarkers to identify relevant candidate genes; molecular events can be linked with phenotypical changes that have initiated them. In this way, adverse outcomes typical of disease classification, even in the absence of usual visual indicators in use for diagnosis *via* pathology, can be revealed. Controlled perturbations can be introduced to provide prototypical settings to develop methodologies suitable for a broad range of biomedical applications (Kriete & Boyce 2005). High-throughput FCM (Edwards *et al*. 2004) or flow and image hybrid systems (George *et al*. 2004) as well as chip-based flow systems (Palkova *et al*. 2004; Wu *et al*. 2004), cellular genomics (Taylor *et al*. 2004), cellular proteomics by immunophenotyping (Maynadié *et al*. 2002; Casasnovas *et al*. 2003; Valet *et al*. 2003; Habib & Finn 2006) or chemical cytometry (Wu *et al*. 2004; Arkhipov *et al*. 2005), as well as cellular metabolomics (Dovichi & Hu 2003) constitute further facets of recent extensions in molecular cytometry.

Areas of research and diagnosis, with the demand to measure 'virtually anything' in the cell include immunophenotyping (Perfetto *et al*. 2004; Pugh-Bernard *et al*. 2004; Miller 2005; Mittag *et al*. 2005; Gerstner *et al*. 2006a), red blood cell analysis (Arndt & Garratty 2004; Greve *et al*. 2004), rare cell detection (Bocsi *et al*. 2004), characterization in the case of stem cells (Bou-Gharios *et al*. 2004; Camargo *et al*. 2004; Jovcic *et al*. 2004; Levering *et al*. 2004; Lovell & Mathur 2004; Rashid *et al*. 2004; Rippon & Bishop 2004) and residual tumour cells (Szaniszlo *et al*. 2004, 2006; Shen & Price 2006), histopathology and drug discovery (Van Osta *et al*. 2006; Van Osta 2006). Systemic analysis is also a prerequisite for predictive medicine by genomics, proteomics and cytomics (Valet *et al*. 2003; Valet & Hoeffkes 2004; Tarnok *et al*. 2006).

Concepts in systemic tumour cell analysis

System-wide single-cell analysis leads to the identification and pinpointing of cell subsets with specific phenotypes and functions (Perfetto *et al*. 2004; Mittag *et al*. 2005). In genomic and proteomic profiling, signals from rare cells may be lost in the background (Szaniszlo *et al*. 2004). Contaminant cancer cells in autologous transplant tissue can cause relapse if their frequency remains unknown. If cells are in suspension, to enrich or deplete specific subtypes is relatively easily satisfied by electrostatic or mechanic cell sorting. Nowadays also, high-throughput technologies for mechanical or optical cell isolation by SBC are available. LEAP™ technology (laser-enabled analysis and processing; Cyntellect Inc., San Diego, CA, USA) offers a unique combination of capabilities in cell purification and selective macromolecule delivery (opto-injection) (Szaniszlo *et al*. 2006). A method capable of removing all contaminant cells, detected by cytomic analyses with a high probability, is combined laser ablation with automated microscopy (Shen & Price 2006). Thus, complete ablation by automated high-content image cytometry may become feasible in combination with pre-therapeutic cytomic analysis of unique cellular expression and/or morphological characteristic patterns (Valet & Hoeffkes 2004; Tarnok *et al*. 2006).

Currently, subcellular location patterns are routinely determined by visual inspection of fluorescence microscope images. In the future, however, automated interpretation of subcellular patterns in fluorescence microscope images for location proteomics (Murphy 2005) and toponomics (Schubert 2003) will become available for mapping and for deciphering functional molecular networks of proteins directly in a cell or a tissue. Proteomics, the large-scale

identification and characterization of one or more signalling cascades expressed in a given cell type, has become a major area of cancer research. In addition to information on protein sequence, structure and expression levels, knowledge of a protein's subcellular location is essential to a complete understanding of its functions (Murphy 2005). Multi-epitope ligand cartography allows colocalization of a large number of proteins in one sample (morphologically intact routinely fixed cells or tissue) with, at present, analysis of up to 18 different cell proteins in the one specimen (Schubert 2003, 2006) with the potential to colocalize hundreds of molecular components. Topologically organized protein networks, in proteomes *in situ*, obey unique protein-colocalization and antico-localization code. Combining diverse data streams across different levels of biological observation such as molecular, cellular and clinical chemistry responses supports a system-wide diagnostic approach. Automated, computerized, microscope-based data extraction and analyses lay the foundation for extracting and correlating measurements, characterizing the functional relationships of spatial localization and protein activation with features of cell migration such as velocity, polarization, protrusion, retraction and mitosis (Debeir *et al*. 2004; Shen *et al*. 2006).

Computational approaches to multicellular assemblies have reached a stage where they may contribute to unveiling the processes that underlie organization of tissues and multicellular aggregates (Galle *et al*. 2006). For quantitative prediction, the control of the biophysical and cell biological parameters on the molecular scale should be known. Here, SBC contributes by permitting tracking the fate of cells and other tissue subunits in time and space, and verifying the organization processes predicted by mathematical models.

Quality control and standardization in systemic analysis

Nowadays, several instruments that have been built around the principle of cytometry are commercially available, with innovative technological developments and software improving the analytical capabilities. Recent expansion in the areas of organic and inorganic fluorescent dyes has greatly facilitated the approach of highly multiplex fluorescence detection for polychromatic (Perfetto *et al*. 2004; Mittag *et al*. 2005; Bocsi *et al*. 2006) and hyperchromatic cytometry (Mittag *et al*. 2006; Schubert 2006), revealing new cell phenotypes with discrete expression patterns. Spectral imaging simplifies and enriches the extraction of morphological and molecular information (Levenson 2006) with user-friendly instrumentation (Ecker *et al*. 2004). The resulting data can be exploited using the spectral content only, or with novel tools, both spectral and spatial information can be combined in the analytical process, using machine-learning approaches for optimization. The next step is to perform hyperspectral fluorescence imaging for *in vivo* detection of malignant tissues and cells (Chung *et al*. 2005, 2006; Zavattini *et al*. 2006) and a combination of these with additional *in vivo* imaging modalities (Ntziachristos *et al*. 2005). These technology combinations may further help to unravel processes in tumour development and tumour cell migration *in vivo* (Wolf *et al*. 2003; Wolf & Friedl 2006).

Object-orientated image analysis could provide detailed and reliable analysis of cellular image data (Baatz *et al*. 2006), and quantitative analysis of polychromatic stained cells in tissue will serve as basis for medical diagnosis and prediction of disease in forthcoming years (Hood *et al*. 2004; Valet *et al*. 2004; Lenz *et al*. 2005). Visualization is a major problem associated with huge inter-dependent data sets. Therefore, alternative and easy-to-handle strategies for data visualization as well as data meta-evaluation (population analysis, cross-correlation, co-expression analysis) are needed (Streit *et al*. 2006), aimed to facilitate human comprehension of complex data.

To collect the most detailed information from single cells standardization must be enabled for:

1 quantification of as many individual components of signalling pathways at the single-cell level;
2 quantitative spatial analysis of intra-cellular structures and inter-relationships of components;
3 quantification of whole-cell function properties;
4 quantification of the inter-relationships between cells.

In principle, all (optical) manipulation affecting fluorescence emission are contradictory to cytometric analysis as measured fluorescence intensities are modified and do not strictly represent antigen expression levels (Mittag *et al*. 2006). Although configuration of various instruments appears to be unique, optical principles are very similar, enabling a number of tests for the evaluation and standardization of equipment performance (Gratama *et al*. 1998; Lerner & Zucker 2004; Zucker 2006). Minimization of sample volume, of necessity in clinical diagnosis and predictive medicine, is facilitated in cytometric techniques (Valet & Hoeffkes 2004; Valet *et al*. 2004; Lenz *et al*. 2005; Valet 2005a,b,c) and is applicable for various types of cancer (Gerstner *et al*. 2003, 2004b, 2005a,b, 2006b; Valet *et al*. 2003).

CONCLUSION

To apply the aspects of cytometry for cytomic analysis, as standard technology in future new instruments, new reagents are required and new computational solutions must be achieved. Instruments are required that enable automated staining, analysis, and (photo)manipulation. Today, several instruments are under development that can at least partially fulfill this demand (Jager *et al*. 2003; Schubert 2006). New small detection molecules such as RNA aptamers (Ulrich *et al*. 2004; Martins *et al*. 2005) are essential to reduce sterical hindrance in highly multiplex analysis. Finally, there is an urgent need for solutions to combine, analyse and document the tremendous amount of data collected by hyperchromatic analysis (Chen *et al*. 2006; Schubert 2006; Streit *et al*. 2006).

The possibility of analysis of genomic and proteomic properties and intra-cellular distributions of whole-cell populations, at the single-cell level in their natural environment, is important if we are to unravel the complexity of cells and cell systems. It opens the way to better understanding of complex processes in health and disease and could be an important tool for predictive and preventative medicine. Cytomics and systems biology can provide information of present tissue status and diagnosis, and consequently promotes the possibility of individualized therapy as a general practice in medicine (Farkas 2003; Hood *et al*. 2004; Valet 2005a,b). Multiparametric and non-invasive analysis of living cells by FCM will elucidate crucial regulative mechanisms within molecular systems, an essential prerequisite for diagnostic interpretation of (co)expression profiles and with significant contribution to the identification of specific drug targets. In contrast to lysate based (functional) techniques, multicolour FCM allows more quantitative simultaneous observations of multiple interactions and/or signalling molecules, in many thousands of individual cells. Multiplex analysis of cells in suspension is nowadays limited by hardware (for example, the optical systems of FCM) as a basis for separation and to detect individual discrete (fluorescent) signals; however, it is yet the most powerful tool for analysis of cell function and dynamics. Cytomics by novel systems can thus be conceived as a joint cross-disciplinary effort for cytomics, systems biology and high-throughput–orientated research for basic, clinical and industrial scientists (Hood *et al*. 2004; Tarnok 2004; Valet & Tarnok 2004; Kriete & Boyce 2005; Valet 2005a,b,c).

Future instruments and/or work flow will enable the analysis of cellular contexts at the single-cell level for the whole organism over high-throughput single-cell analysis in tissues

(tissomics, toponomics). This can be combined with single-cell high-content metabolic investigation for lipidomics (Wenk 2005; Werner *et al*. 2006) and metabolomics, intra-cellular location proteomics down to single-cell proteomics and genomics. Consequently, this will lead to merging of genomics, cytomics and proteomics with systems analysis (Bocharov 2005; Bernas *et al*. 2006). Combined with data pattern analysis, these approaches will lead to complete characterization of the cytome (Valet & Tarnok 2004; Valet 2005b,c) with the detection of new cell types, cellular functions and inter-relationships and imminent implications for individualized risk assessment (Valet & Tarnok 2003; Hood *et al*. 2004; Lenz *et al*. 2005).

ACKNOWLEDGEMENTS

We thank Dr. Simone Diermeier (Institute of Pathology, University of Regensburg) for her support with this manuscript.

REFERENCES

Abramovitz M, Leyland-Jones B (2006) A systems approach to clinical oncology: focus on breast cancer. *Proteome Sci*. **4**, 5.

Alberghina L, Chiaradonna F, Vanoni M (2004) Systems biology and the molecular circuits of cancer. *Chembiochem*. **5**, 1322–1333.

Arkhipov SN, Berezovski M, Jitkova J, Krylov SN (2005) Chemical cytometry for monitoring metabolism of a Ras-mimicking substrate in single cells. *Cytometry A* **63A**, 41–47.

Arndt PA, Garratty G (2004) Flow cytofluorometric analysis in red blood cell immunology. *Transfus*. *Med*. *Hemother*. **31**, 163–174.

Baatz M, Arini N, Schäpe A, Binnig G, Linssen B (2006) Object-oriented image analysis for high content screening: detailed quantification of cells and sub-cellular structures with the Cellenger software. *Cytometry A* **69A**, 652–658.

Bernas T, Gregori G, Asem EK, Robinson JP (2006) Integrating cytomics and proteomics. *Mol*. *Cell*. *Proteomics* **5**, 2–13.

Bocharov G (2005) Understanding complex regulatory systems: integrating molecular biology and systems analysis. *Transfus*. *Med*. *Hemother*. **32**, 304–321.

Bocsi J, Varga VS, Molnar B, Sipos F, Tulassay Z, Tarnok A (2004) Scanning fluorescent microscopy analysis is applicable for absolute and relative cell frequency determinations. *Cytometry A* **61A**, 1–8.

Bocsi J, Lenz D, Mittag A, Varga VS, Molnar B, Tulassay Z, Sack U, Tarnok A (2006) Automated four-color analysis of leukocytes by scanning fluorescence microscopy using quantum dots. *Cytometry A* **69A**, 131–134.

Bou-Gharios G, Ponticos M, Rajkumar V, Abraham D (2004) Extra-cellular matrix in vascular networks. *Cell Prolif*. **37**, 207–220.

Brockhoff G, Heiss P, Schlegel J, Hofstaedter F, Knuechel R (2001) Epidermal growth factor receptor, c-erbB2 and c-erbB3 receptor interaction, and related cell cycle kinetics of SK-BR-3 and BT474 breast carcinoma cells. *Cytometry* **44**, 338–348.

Camargo FD, Chambers SM, Goodell MA (2004) Stem cell plasticity: from transdifferentiation to macrophage fusion. *Cell Prolif*. **37**, 55–65.

Casasnovas RO, Slimane FK, Garand R, Faure GC, Campos L, Deneys V, Bernier M, Falkenrodt A, Lecalvez G, Maynadié M, Béné MC (2003) Immunological classification of acute myeloblastic leukemias: relevance to patient outcome. *Leukemia* **17**, 515–527.

Celis JE, Moreira JM, Gromova I, Cabezon T, Ralfkiaer U, Guldberg P, Straten PT, Mouridsen H, Friis E, Holm D, Rank F, Gromov P (2005) Towards discovery-driven translational research in breast cancer. *FEBS J*. **272**, 2–15.

Chassagnole C, Jackson RC, Hussain N, Bashir L, Derow C, Savin J, Fell DA (2006) Using a mammalian cell cycle simulation to interpret differential kinase inhibition in anti-tumour pharmaceutical development. *Biosystems* **83**, 91–97.

Chen X, Velliste M, Murphy RF (2006) Automated interpretation of subcellular patterns in fluorescence microscope images for location proteomics. *Cytometry A* **69A**, 631–640.

Chung A, Wachsmann-Hogiu S, Zhao T, Xiong Y, Joseph A, Farkas DL (2005) Advanced optical imaging requiring no contrast agents – a new armamentarium for medicine and surgery. *Curr. Surg.* **62**, 365–370.

Chung A, Karlan S, Lindsley E, Wachsmann-Hogiu S, Farkas DL (2006) *In vivo* cytometry: a spectrum of possibilities. *Cytometry A* **69A**, 142–146.

Debeir O, Camby I, Kiss R, Van Ham P, Decaestecker C (2004) A model-based approach for automated *in vitro* cell tracking and chemotaxis analyses. *Cytometry A* **60A**, 29–40.

Diermeier S, Horvath G, Knuechel-Clarke R, Hofstaedter F, Szollosi J, Brockhoff G (2005) Epidermal growth factor receptor coexpression modulates susceptibility to Herceptin in HER2/neu overexpressing breast cancer cells via specific erbB-receptor interaction and activation. *Exp. Cell Res.* **304**, 604–619.

Dovichi NJ, Hu S (2003) Chemical cytometry. *Curr. Opin. Chem. Biol.* **7**, 603–608.

Ecker RC, Steiner GE (2004) Microscopy-based multicolor tissue cytometry at the single-cell level. *Cytometry A* **59A**, 182–190.

Ecker RC, Tarnok A (2005) Cytomics goes 3D: toward tissomics. *Cytometry A* **65A**, 1–3.

Ecker RC, De Martin R, Steiner GE, Schmid JA (2004) Application of spectral imaging microscopy in cytomics and fluorescence resonance energy transfer (FRET) analysis. *Cytometry A* **59A**, 172–181.

Edwards BS, Oprea T, Prossnitz ER, Sklar LA (2004) Flow cytometry for high-throughput, high-content screening. *Curr. Opin. Chem. Biol.* **8**, 392–398.

Elkin EB, Weinstein MC, Winer EP, Kuntz KM, Schnitt SJ, Weeks JC (2004) HER-2 testing and trastuzumab therapy for metastatic breast cancer: a cost-effectiveness analysis. *J. Clin. Oncol.* **22** (5), 854–863.

Farkas DL (2003) Invention and commercialization in optical bioimaging. *Nat. Biotechnol.* **21**, 1269–1271.

Galle J, Aust G, Schaller G, Beyer T, Drasdo D (2006) Individual cell-based models of the spatial-temporal organization of multi-cellular systems – achievements and limitations. *Cytometry A* **69A**, 704–710.

George TC, Basiji DA, Hall BE, Lynch DH, Ortyn WE, Perry DJ, Seo MJ, Zimmerman CA, Morrissey PJ (2004) Distinguishing modes of cell death using the ImageStream multispectral imaging flow cytometer. *Cytometry A* **59A**, 237–245.

Gerstner AOH, Müller AK, Machlitt J, Tárnok A, Tannapfel A, Weber A, Bootz F (2003) Slide-based cytometry for predicting malignancy in solid salivary gland tumors by fine needle aspirate biopsies. *Cytometry B Clin. Cytom.* **53B**, 20–25.

Gerstner AO, Trumpfheller C, Racz P, Osmancik P, Tenner-Racz K, Tárnok A (2004a) Quantitative histology by multicolor slides-based cytometry. *Cytometry A* **59A**, 210–219.

Gerstner AO, Gutsche M, Bucheler M, Machlitt J, Emmrich F, Sommerer F, Tarnok A, Bootz F (2004b) Eosinophilia in nasal polyposis: its objective quantification and clinical relevance. *Clin. Exp. Allergy* **34**, 65–70.

Gerstner AO, Thiele A, Tarnok A, Machlitt J, Oeken J, Tannapfel A, Weber A, Bootz F (2005a) Preoperative detection of laryngeal cancer in mucosal swabs by slide-based cytometry. *Eur. J. Cancer* **41**, 445–452.

Gerstner AO, Tarnok A, Bootz F (2005b) Slide-based multi-parametric cytometry in ENT. Perspectives for the clinic and research. *HNO* **53**, 134–141.

Gerstner AO, Mittag A, Laffers W, Dahnert I, Lenz D, Bootz F, Bocsi J, Tarnok A (2006a) Comparison of immunophenotyping by slide-based cytometry and by flow cytometry. *J. Immunol. Methods* **311**, 130–138.

Gerstner AOH, Thiele A, Tárnok A, Tannapfel A, Weber A, Bootz F (2006b) Prediction of upper aero-digestive tract cancer by slide-based cytometry. *Cytometry A* **69A**, 582–587.

Gratama JW, D'hautcourt JL, Mandy F, Rothe G, Barnett D, Janossy G, Papa S, Schmitz G, Lenkei R (1998) Flow cytometric quantitation of immunofluorescence intensity: problems and perspectives. European Working Group on Clinical Cell Analysis. *Cytometry* **33**, 166–178.

Greve B, Valet G, Humpe A, Tonn T, Cassens U (2004) Flow cytometry in transfusion medicine: development, strategies and applications. *Transfus. Med. Hemother.* **31**, 152–161.

Habib LK, Finn WG (2006) Unsupervised immunophenotypic profiling of chronic lymphocytic leukemia. *Cytometry B Clin. Cytom.* **70B**, 124–135.

Hood L, Heath JR, Phelps ME, Lin B (2004) Systems biology and new technologies enable predictive and preventative medicine. *Science* **306**, 640–643.

Hornberg JJ, Bruggeman FJ, Westerhoff HV, Lankelma J (2006) Cancer: a systems biology disease. *Biosystems* **83**, 81–90.

Jager S, Garbow N, Kirsch A, Preckel H, Gandenberger FU, Herrenknecht K, Rudiger M, Hutchinson JP, Bingham RP, Ramon F, Bardera A, Martin J (2003) A modular, fully integrated ultra-high-throughput screening system based on confocal fluorescence analysis techniques. *J. Biomol. Screen.* **8**, 648–659.

Jones RB, Gordus A, Krall JA, MacBeath G (2006) A quantitative protein interaction network for the ErbB receptors using protein microarrays. *Nature* **439**, 168–174.

Jovcic G, Bugarski D, Petakov M, Krstic A, Vlaski M, Stojanovic N, Milenkovic P (2004) *In vivo* effects of interleukin-17 on haematopoietic cells and cytokine release in normal mice. *Cell Prolif.* **37**, 401–412.

Kantor AB, Alters SE, Cheal K, Dietz LJ (2004) Immune systems biology: immunoprofiling of cell and molecules. *Biotechniques* **36**, 520–524.
Khalil IG, Hill C (2005) Systems biology for cancer. *Curr. Opin. Oncol.* **17**, 44–48.
Kitano H (2002) Computational systems biology. *Nature* **420**, 206–210.
Kriete A (2005) Cytomics in the realm of systems biology. *Cytometry A* **68A**, 19–20.
Kriete A, Boyce K (2005) Automated tissue analysis-a bioinformatics perspective. *Methods Inf. Med.* **44**, 32–37.
Kriete A, Boyce K, Love B (2006) System-wide analysis of hepatotoxicological responses: tissomics is key. *Cytometry A* **69A**, 612–619.
Lenz D, Barten MJ, Hiller S, Tarnok A, Sack U (2005) Regenerative and predictive medicine of cardiovascular disease: the 9th Leipziger Workshop and the 2nd International Workshop on slide-based cytometry. *Cytometry A* **64A**, 110–114.
Lerner JM, Zucker RM (2004) Calibration and validation of confocal spectral imaging systems. *Cytometry A* **62A**, 8–34.
Levenson RM (2006) A spectral imaging perspective on cytomics. *Cytometry A* **69A**, 592–600.
Levering WHBM, Sutherland DR, Keeney M, Kraan J, Gratama JW (2004) Haematopoietic stem and progenitor cells: enumeration, phenotypic characterisation, and clinical applications. *Transfus. Med. Hemother* **31**, 341–352.
Liu ET, Kuznetsov VA, Miller LD (2006) In the pursuit of complexity: systems medicine in cancer biology. *Cancer Cell* **9**, 245–247.
Lottner C, Schwarz S, Diermeier S, Hartmann A, Knuechel R, Hofstaedter F, Brockhoff G (2005) Simultaneous detection of HER2/neu gene amplification and protein overexpression in paraffin-embedded breast cancer. *J. Pathol.* **205**, 577–584.
Lovell MJ, Mathur A (2004) The role of stem cells for treatment of cardiovascular disease. *Cell Prolif.* **37**, 67–87.
Martins AH, Resende RR, Majumder P, Faria M, Casarini DE, Tarnok A, Colli W, Pesquero JB, Ulrich H (2005) Neuronal differentiation of P19 embryonal carcinoma cells modulates kinin B2 receptor gene expression and function. *J. Biol. Chem.* **280**, 19576–19586.
Maynadié M, Picard F, Husson B, Chatelain B, Cornet Y, Le Roux G, Campos L, Dromelet A, Lepelley P, Jouault H, Imbert M, Rosenwadj M, Vergé V, Bissière P, Raphaël M, Béné MC, Feuillard J, Groupe d'Etude Immunologique des Leucemies (GEIL) (2002) Immunophenotype clustering of myelodysplastic syndromes. *Blood.* **100**, 2349–2356.
Megyeri A, Bacso Z, Shields A, Eliason JF (2005) Development of a stereological method to measure levels of fluoropyrimidine metabolizing enzymes in tumor sections using laser scanning cytometry. *Cytometry A* **64A**, 62–71.
Miller JFAP (2005) Principles of immunological tolerance. *Transfus. Med. Hemother.* **32**, 322–331.
Mittag A, Lenz D, Gerstner AOH, Sack U, Steinbrecher M, Koksch M, Raffael A, Bocsi J, Tárnok A (2005) Polychromatic eight-color slide-based cytometry for the characterization of NK and NK-T subsets. *Cytometry A* **65A**, 103–115.
Mittag A, Lenz D, Gerstner AOH, Tárnok A (2006) Hyperchromatic cytometry principles for cytomics using slide-based cytometry. *Cytometry A* **69A**, 691–703.
Mosch B, Mittag A, Lenz D, Arendt T, Tarnok A (2006) Laser scanning cytometry in human brain slices. *Cytometry A* **69A**, 135–138.
Murphy RF (2005) Cytomics and location proteomics: automated interpretation of subcellular patterns in fluorescence microscope images. *Cytometry A* **67A**, 1–3.
Ntziachristos V, Ripoll J, Wang LV, Weissleder R (2005) Looking and listening to light: the evolution of whole-body photonic imaging. *Nat. Biotechnol.* **23**, 313–320.
Palkova Z, Vachova L, Valer M, Preckel T (2004) Single-cell analysis of yeast, mammalian cells, and fungal spores with a microfluidic pressure-driven chip-based system. *Cytometry A* **59A**, 246–253.
Perfetto SP, Chattopadhyay PK, Roederer M (2004) Seventeen-colour flow cytometry: unravelling the immune system. *Nat. Rev. Immunol.* **4**, 648–655.
Perlman ZE, Slack MD, Feng Y, Mitchison TJ, Wu LF, Altschuler SJ (2004) Multidimensional drug profiling by automated microscopy. *Science* **306**, 1194–1198.
Pugh-Bernard A, Cappione A, Anolik J, Sanz I (2004) From cold-agglutinin disease to systemic lupus erythematosus: lessons in human B-cell tolerance and its breakdown. *Transfus. Med. Hemother.* **31**, 84–90.
Rashid ST, Salacinski HJ, Fuller BJ, Hamilton G, Seifalian AM (2004) Engineering of bypass conduits to improve patency. *Cell Prolif.* **37**, 351–366.
Richter M, Zhang H (2005) Receptor-targeted cancer therapy. *DNA Cell Biol.* **24**, 271–282.
Rippon HJ, Bishop AE (2004) Embryonic stem cells. *Cell Prolif.* **37**, 23–34.
Sachs K, Perez O, Pe'er D, Lauffenburger DA, Nolan GP (2005) Causal protein-signaling networks derived from multiparameter single-cell data. *Science* **308** (5721), 523–529.
Schubert W (2003) Topological proteomics, toponomics, MELK-technology. *Adv. Biochem. Eng. Biotechnol.* **83**, 189–209.
Schubert W (2006) Exploring molecular networks directly in the cell. *Cytometry A* **69A**, 109–112.

Schulze WX, Deng L, Mann M (2005) Phosphotyrosine interactome of the ErbB-receptor kinase family. *Mol. Syst. Biol.* **1**, No.2005.0008 Msb4100012-E1-E8.

Shen F, Price JH (2006) Toward complete laser ablation of melanoma contaminant cells in a co-culture outgrowth model via image cytometry. *Cytometry A* **69A**, 573–581.

Shen F, Hodgson L, Rabinovich A, Pertz O, Hahn K, Price JH (2006) Functional proteometrics for cell migration. *Cytometry A* **69A**, 563–572.

Spencer SL, Berryman MJ, Garcia JA, Abbott D (2004) An ordinary differential equation model for the multistep transformation to cancer. *J. Theor. Biol.* **231**, 515–524.

Steiner GE, Ecker RC, Kramer G, Stockenhuber F, Marberger MJ (2000) Automated data acquisition by confocal laser scanning microscopy and image analysis of triple stained immunofluorescent leukocytes in tissue. *J. Immunol. Methods* **237**, 39–50.

Streit M, Ecker RC, Österreicher K, Steiner GE, Bischof H, Bangert C, Kopp T, Rogojanu R (2006) 3D parallel coordinate systems – a new data visualization method in the context of microscopy-based multicolor tissue cytometry (MMTC). *Cytometry A* **69A**, 601–611.

Szaniszlo P, Wang N, Sinha M, Reece LM, Van Hook JW, Luxon BA, Leary JF (2004) Getting the right cells to the array: gene expression microarray analysis of cell mixtures and sorted cells. *Cytometry A* **59A**, 191–202.

Szaniszlo P, Rose WA, Wang N, Reece LM, Tsulaia TV, Hanania EG, Elferink CJ, Leary JF (2006) Scanning cytometry with a LEAP: laser-enabled analysis and processing of live cells in situ. *Cytometry A* **69A** (7), 641–651.

Tabach Y, Milyavsky M, Shats I, Brosh R, Zuk O, Yitzhaky A, Mantovani R, Domany E, Rotter V, Pilpel Y (2005) The promoters of human cell cycle genes integrate signals from two tumor suppressive pathways during cellular transformation. *Mol. Syst. Biol.* **1**, No.2005.0022 Msb4100030-E1-E15.

Tarnok A (2004) New technologies for the human cytome project. *J. Biol. Regul. Homeost. Agents* **18**, 92–95.

Tarnok A (2006) Slide-based cytometry for cytomics – A minireview. *Cytometry A* **69A** (7), 555–562.

Tarnok A, Gerstner AO (2002) Clinical applications of laser scanning cytometry. *Cytometry* **50** (3), 133–143.

Tarnok A, Valet G, Emmrich F (2006) Systems biology and clinical cytomics: The 10th Leipziger Workshop and the 3rd International Workshop on Slide-Based Cytometry, Leipzig, Germany, April 2005. *Cytometry A* **69A**, 36–40.

Taylor TB, Nambiar PR, Raja R, Cheung E, Rosenberg DW, Anderegg B (2004) Microgenomics: identification of new expression profiles via small and single-cell sample analysis. *Cytometry A* **59A**, 254–261.

Uetz P, Finley RL Jr (2005) From protein networks to biological systems. *FEBS Lett.* **579**, 1821–1827.

Uetz P, Stagljar I (2006) The interactome of human EGF/ErbB receptors. *Mol. Syst. Biol.* **2**, 2006.0006 Msb4100048-E1-E2.

Ulrich H, Martins AH, Pesquero JB (2004) RNA and DNA aptamers in cytomics analysis. *Cytometry A* **59A**, 220–231.

Valet G (2003) Past and present concepts in flow cytometry: a European perspective. *J. Biol. Regul. Homeost. Agents* **17**, 213–222.

Valet G (2005a) Cytomics: an entry to biomedical cell systems biology. *Cytometry A* **63A**, 67–68.

Valet G (2005b) Cytomics, the human cytome project and systems biology: top-down resolution of the molecular biocomplexity of organisms by single cell analysis. *Cell Prolif.* **38**, 171–174.

Valet G (2005c) Human cytome project, cytomics, and systems biology: the incentive for new horizons in cytometry. *Cytometry A* **64A**, 1–2.

Valet G, Tarnok A (2003) Cytomics in predictive medicine. *Cytometry B Clin. Cytom.* **53B**, 1–3.

Valet G, Tarnok A (2004) Potential and challenges of a human cytome project. *J. Biol. Regul. Homeost. Agents* **18**, 87–91.

Valet G, Hoeffkes HG (2004) Data pattern analysis for the individualised pretherapeutic identification of high-risk diffuse large B-cell lymphoma (DLBCL) patients by cytomics. *Cytometry A* **59A**, 232–236.

Valet G, Repp R, Link H, Ehninger G, Gramatzki M, SHG-AML Study Group (2003) Pretherapeutic identification of high-risk acute myeloid leukemia (AML) patients from immunophenotypic, cytogenetic, and clinical parameters. *Cytometry B Clin. Cytom.* **53B**, 4–10.

Valet G, Leary JF, Tarnok A (2004) Cytomics – new technologies: towards a human cytome project. *Cytometry A* **59A**, 167–171.

Van Leeuwen IM, Byrne HM, Jensen OE, King JR (2006) Crypt dynamics and colorectal cancer: advances in mathematical modelling. *Cell Prolif.* **39**, 157–181.

Van Osta P (2006) Extracting quantitative information from tissue – an industrial perspective. *Cytometry A* **69A**, 588–591.

Van Osta P, Ver Donck K, Bols L, Geysen J (2006) Cytomics and drug discovery. *Cytometry A* **69A**, 117–118.

Varga VS, Bocsi J, Sipos F, Csendes G, Tulassay Z, Molnar B (2004) Scanning fluorescent microscopy is an alternative for quantitative fluorescent cell analysis. *Cytometry A* **60A**, 53–62.

Vereb G, Matko J, Szollosi J (2004) Cytometry of fluorescence resonance energy transfer. *Methods Cell Biol.* **75**, 105–152.

Wenk MR (2005) The emerging field of lipidomics. *Nat. Rev. Drug Discov.* **4**, 594–610.

Werner T, Liebisch G, Grandl M, Schmitz G (2006) Evaluation of a high-content screening fluorescence-based assay analyzing the pharmacological modulation of lipid homeostasis in human macrophages. *Cytometry A* **69A**, 200–202.

Wiley HS, Shvartsman SY, Lauffenburger DA (2003) Computational modeling of the EGF-receptor system: a paradigm for systems biology. *Trends Cell Biol*. **13**, 43–50.

Wolf K, Friedl P (2006) Molecular mechanisms of cancer cell invasion and plasticity. *Br. J. Dermatol*. **154** (Suppl. 1), 11–15.

Wolf K, Mazo I, Leung H, Engelke K, Von Andrian UH, Deryugina EI, Strongin AY, Brocker EB, Friedl P (2003) Compensation mechanism in tumor cell migration: mesenchymal-amoeboid transition after blocking of pericellular proteolysis. *J. Cell Biol*. **160**, 267–277.

Wu H, Wheeler A, Zare RN (2004) Chemical cytometry on a picoliter-scale integrated microfluidic chip. *PNAS* **101**, 12809–12813.

Yarden Y, Sliwkowski MX (2001) Untangling the ErbB signalling network. *Nat. Rev. Mol. Cell Biol*. **2**, 127–137.

Zavattini G, Vecchi S, Mitchell G, Weisser U, Leahy RM, Pichler BJ, Smith DJ, Cherry SR (2006) A hyperspectral fluorescence system for 3D *in vivo* optical imaging. *Phys. Med. Biol*. **51**, 2029–2043.

Zucker RM (2006) Quality assessment of confocal microscopy slide-based systems: performance. *Cytometry A* **69A**, 659–676.