Edited by Valery V. Tuchin

WILEY-VCH

Advanced Optical Flow Cytometry

Methods and Disease Diagnoses

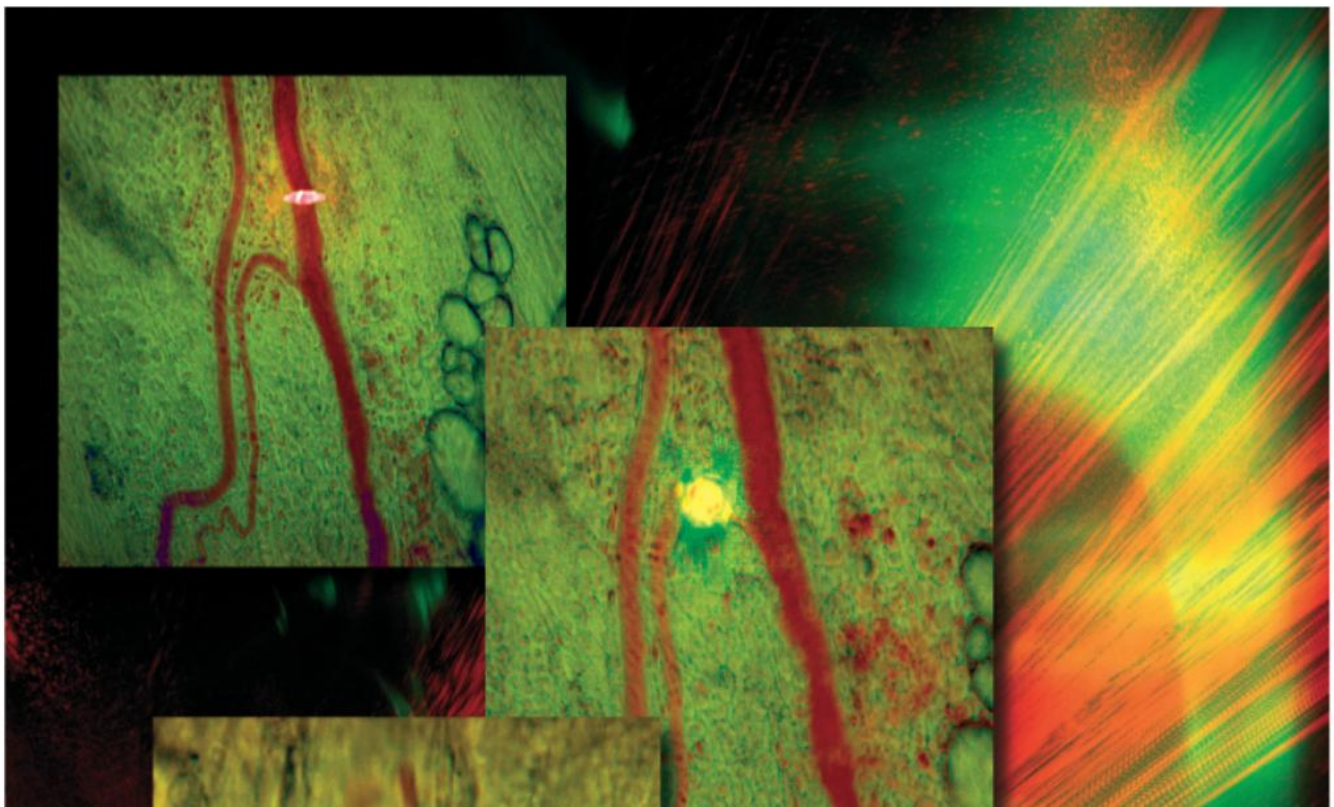

Table of Contents

Related Titles

Lakshmipathy, U., Chesnut, J. D., Thyagarajan, B.
Emerging Technology Platforms for Stem Cells
2009
506 pages
Hardcover
ISBN: 978-0-470-14693-4

Wolfbeis,O. S. (ed.)
Fluorescence Methods and Applications
Spectroscopy, Imaging, and Probes
2008
452 pages
Softcover
ISBN: 978-1-57331-716-0

Murphy, D. B.
Fundamentals of Light Microscopy and Electronic Imaging
2008
E-Book
ISBN: 978-0-470-24202-5

Prasad, P. N.
Introduction to Biophotonics
2004
E-Book
ISBN: 978-0-471-46539-3

Durack, G., Robinson, J. P. (eds.)
Emerging Tools for Single-Cell Analysis

Advances in Optical Measurement Technologies
2002
Online Book Wiley Interscience
ISBN: 978-0-471-22484-6

Givan, A. L.
Flow Cytometry
First Principles
296 pages
2001
Softcover
ISBN: 978-0-471-38224-9

Edited by Valery V. Tuchin

Advanced Optical Flow Cytometry

Methods and Disease Diagnoses

WILEY-VCH Verlag GmbH & Co. KGaA

The Editor

Prof. Valery V. Tuchin

Saratov State University

Optics and Biophotonics

Saratov, Russian Feeration

tuchinvv@mail.ru

Cover

Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library.

Bibliographic information published by the Deutsche Nationalbibliothek

The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available on the Internet at <http://dnb.d-nb.de>.

ISBN: 978-3-527-40934-1

ePDF: 978-3-527-63430-9

oBook: 978-3-527-63428-6

ePub: 978-3-527-63429-3

Mobi: 978-3-527-63431-6

Preface

Flow cytometry was invented in the late 1960s, and since then the flow cytometer has become an indispensable tool in modern research and clinical laboratories [1–7]. Beyond the routine usage, new trends can be observed in the development of flow cytometric techniques. The main technological improvements include high-speed sorting, phase-sensitive flow cytometry, multicolor flow cytometry, high-throughput multiplex bead assays, and spectral detection, and it provides the basis for extensive data collection.

Classical flow cytometry (FC) uses an instrument system for making, processing, and displaying one or more measurements on individual cells in flowing cell suspension [1–7]. Cells may be stained with one or more fluorescent dyes specific to cell components of interest, for example, DNA, and fluorescence of each cell is measured as cells one by one rapidly transverse the excitation beam (laser or mercury arc lamp). Fluorescence provides a quantitative measure of various biochemical and biophysical properties of the cell. Other measurable optical parameters, which are applicable to the measurement of cell size, shape, density, granularity, and stain uptake, include light absorption, light scattering, and polarization degree.

Numerous clinical and research applications, especially in anatomic pathology for detection and study of malignant lesions, use the so-called image cytometry. This technique encompasses morphometry and densitometry as measuring techniques, and neural networks and expert systems for processing of collected data.

Another cytometric technique is the microscope-based *laser scanning cytometry*, which allows one to make

fluorescence measurements and topographic analysis on individual cells. Laser-induced fluorescence of labeled cellular specimens is detected using multiple discrete wavelengths, and the spatially resolved data are processed to quantify cell proliferation, apoptosis, gene expression, protein transport, and other cellular processes. For instance, confocal microscopy and two-photon imaging techniques are able to detect fluorescently labeled cells not only *in vitro* but also *in vivo* [8].

Improvements in image cytometric techniques speeded up in the last two decades, applying more and more sensitive detectors and introducing nonlinear optics (two photon excitation) for lifetime measurements (fluorescence lifetime imaging microscopy, FLIM). The latest developments were able to break the diffraction limit as in the scanning near-field optical microscopy (SNOM), total internal reflection microscopy (TIRFM), fluorescence resonance energy transfer (FRET), stimulated emission depletion (STED), and 4Pi and multiobjective microscopy.

Conventional FC is currently the method of choice for rapid quantification of cells, but it requires invasive extraction of cells from a living organism and associated procedures (e.g., fluorescence labeling and sorting), which may lead to unpredictable artifacts, and prevents long-term cell monitoring in the native biological environment. Among *in vivo* techniques, both nonoptical (e.g., PET and MRI) and optical (e.g., scattering, fluorescence, confocal, and multiphoton) techniques can be used for visualizing only single static or slowly migrating cells [8–10]. To detect fast moving cells in blood and lymph flows, a number of methods providing *in vivo* FC have been developed [11–21]. In particular, the principle of FC has been adapted to the *in vivo*

monitoring of labeled cells in ear blood vessels, and a few modifications of *in vivo* flow cytometers that are capable of real-time confocal detection of fluorescently labeled cells in both the arterial and venous circulation of small animals, have been built [12–14].

The alternative photothermal (PT) and photoacoustic (PA) techniques for *in vivo* blood FC, which do not require cell labeling and are not sensitive to light scattering and autofluorescence background, have also been recently suggested [15–17]. These techniques have potential application in the study of normal and abnormal cells in their native condition in blood or lymph flows *in vivo*, including molecular imaging, studying the metabolism and pathogenesis of diseases at a cellular level, and monitoring and quantifying metastatic and apoptotic cells and/or their responses to therapeutic interventions (e.g., drug or radiation).

Video microscopy and particle tracking methods adapted and integrated with an ultrahigh-speed imaging camera were used to measure lymph velocities throughout the entire lymphatic contraction cycle in the rat mesentery [18–23]. *In vivo*, label-free, high-speed (up to 10 000 with the potential for 40 000 fps), high-resolution (up to 300 nm) optical imaging of circulating individual erythrocytes, leukocytes, and platelets in fast blood flow has been developed [22]. Different potential applications of *in vivo* digital video microscopy include visualization of circulating cells and their deformability in lymph and blood flows and the study of the kinetics of platelets and leukocyte rolling, with high sensitivity and resolution.

Multiphoton fluorescence flow cytometry and its confocal and fiber-optic modifications hold a great promise for *in vivo* monitoring of multiple circulating cell populations in blood and lymph flows by exciting and

detecting the emission from multiple fluorophores, such as fluorescent proteins and exogenous chromophores, important for multilabeling of cells of interest [24, 25].

There are many books on cytometry published since the 1980s (see, for example, the list on the web site [1]). They could be classified as books on general flow cytometry and cell sorting, clinical cytometry, and microscopic and imaging cytometry. The most recent and comprehensive are Refs [2, 3, 5, 8–10, 26–30]. The book by Shapiro [5] is the fourth edition on classic flow cytometry. This is one of the best textbooks, and covers well the field of practical flow cytometry prior to 2003 well. Recently, two books on flow cytometry and cellular diagnostics have been published in German and French [26, 27]. Both books were written and edited by well-known experts in the field. Karger has published the English translation of the German book edited by Ulrich Sack, Attila Tárnok, and Gregor Rothe, which is a bestseller in German [28]. Practical cytometry protocols have been given in the third edition of the book edited by Michael G. Ormerod [29]. The recent second edition of the book by Wojciech Gorczyca is more clinically and practically oriented [30]. Michael G. Ormerod has also designed an introductory book “to give that knowledge, aiming at people coming to flow cytometry for the first time,” in which all the major applications in mammalian biology are covered [31].

While the above-mentioned books describe clinical diagnostic methods and receipts of their applications, the current book is more research oriented and opens new perspectives in the development of flow cytometry for *in vivo* studies. It contains novel results of basic research on light scattering by different types of cells, which are very important for the improvement of already existing technologies and for designing new technologies in

optical cytometry. The recently invented and fast-moving-to-practice methods of *in vivo* flow cytometry, based on ultrafast video and phase intra-vital microscopy and light scattering, diffraction, speckle, fluorescence, multiphoton, Raman, photothermal, and photoacoustic phenomena, are presented in the book.

In Chapter 1, *Perspectives in Cytometry* by Anja Mittag and Attila Tárnok, in addition to definitions, historical aspects, and the importance of cytometry in the development of biology and medicine, its prospective application as a science and diagnostic tool are discussed – in particular, for comprehensive analyses, on the basis of the simultaneous detection of several parameters, of up to millions of individual cells in one sample.

Chapter 2 by Herbert Schneckenburger *et al.*, *Novel Concepts and Requirements in Cytometry*, presents slide-based cytometry techniques and the concepts of high content screening (HCS) where detailed information is accumulated from a single cell or examination of multicellular spheroids where 3D detection methods are required. These techniques include microscopic setups, fluorescence reader systems, and microfluidic devices with micromanipulation, for example, cell sorting.

In Chapter 3 by Stoyan Tanev *et al.*, *Optical Imaging of Cells with Gold Nanoparticle Clusters as Light Scattering Contrast Agents: A Finite-Difference Time-Domain Approach to the Modeling of Flow Cytometry Configurations*, a brief summary of different formulations of the finite-difference time-domain (FDTD) approach is presented in the framework of its strengths, for cytometry in general and for potential applications in *in vivo* flow cytometry based on light scattering, including nanoscale targets. This chapter focuses on comparison of light scattering by a single biological cell alone under controlled refractive index matching conditions and by

cells labeled using gold nanoparticle clusters. The optical phase contrast microscopy (OPCM) is analyzed as a prospective modality for *in vivo* flow cytometry.

In Chapter 4 by Valeri P. Maltsev *et al.*, *Optics of White Blood Cells: Optical Models, Simulations, Experiments*, a state-of-the-art summary of analytical and numerical simulating methods and experimental approaches for precise description and detection of elastic light scattering from white blood cells (WBCs) are presented. The discussion of the instrumental tools for measurement of light scattering lays emphasis on scanning flow cytometry. This chapter gives some basis for understanding the methods, techniques, and experimental results presented in the following chapters, as it presents solutions for the inverse light-scattering problem to obtain cellular characteristics from light scattering data.

The optical properties of blood are discussed in Chapter 5 by Martina Meinke *et al.*, *Optical Properties of Flowing Blood Cells*. Authors use the transport theory accounting for the multiplicity of light scattering events, where the optical properties of blood are described by the absorption and scattering coefficients and the anisotropy factor. The double integrating sphere measurement technique combined with inverse Monte Carlo simulation is applied for extraction of the optical parameters of undiluted blood. It is shown that the influence of the shear rate and osmolarity have to be taken into account when the blood is prepared *ex vivo* and the physiological environment cannot be ensured.

In Chapter 6, *Laser Diffraction on RBC and Deformability Measurement* by Alexander V. Priezzhev *et al.*, RBC shape variability and deformability as intrinsic properties, their strong relation to RBC aggregation and blood rheology, and the determination of the general hemorheologic

status of human organism are considered. It is shown that laser diffraction can be efficiently applied to quantitatively assess the deformability properties of RBC in the blood of a particular individual. The theoretical basis of diffractometry and implementation of particular experimental techniques to experimental and clinical measurements, as well as potentialities and pitfalls of the technique are discussed.

The principles and fundamentals of flicker spectroscopy as a quantitative tool to measure static and dynamic mechanical properties of composite cell membrane are presented in Chapter 7, *Characterization of Red Blood Cells Rheological and Physiological State Using Optical Flicker Spectroscopy* by Vadim L. Kononenko. These mechanical properties are associated with cell membrane and cytoplasm molecular organization and composition and cell metabolic activity and could be characteristic not only for RBC, but for other blood cell types as well. Microscope-based flicker spectroscopy technique in combination with quantitative phase imaging and fluorescence microscopy can be easy integrated into the slide-based cytometry arrangement. As the author states, the approximate models developed are good, but not enough to reconstruct precisely the details of erythrocyte cell membrane mechanical properties; thus a more advanced theory of flicker spectroscopy is needed.

Chapter 8, *Digital Holographic Microscopy for Quantitative Live Cell Imaging and Cytometry*, by Björn Kemper and Jürgen Schnekenburger, demonstrates the principles and applications of quantitative cell imaging using digital holographic microscopy (DHM). The quantitative phase contrast imaging, cell thickness determination, multifocus imaging, and 2D cell tracking provided by DHM show that it is a suitable method for the label-free characterization of dynamic live cell processes

involving morphological alterations and migration and for the analysis of cells in 3D environments. Examples and illustrations of applicability of the technique in tumor cell biology and for the development of improved systems for drug and toxicity testing are presented.

Chapter 9 by József Bocsi *et al.*, *Comparison of Immunophenotyping and Rare Cell Detection by Slide-Based Imaging Cytometry and by Flow Cytometry*, allows one to get answers on the following questions: are flow cytometry (FC) and slide-based cytometry (SBC) comparable? What is the type of cytometer and analysis technique that should be chosen for the given biological problem to be solved? Answers are illustrated by applying scanning fluorescence microscope (SFM) to determine of CD4/CD8 T cell ratio, laser scanning cytometer (LSC) to multiparametric leukocyte phenotyping and apoptosis analysis on the basis of DNA content measurements, and SFM and LSC to rare and frequent tumor cell detection.

A brief overview and discussion of recent progress in microfluidic flow cytometry, including the main components of full scale flow cytometers, containing systems for fluidic control, optical detection and cell sorting, each of which are being developed into on-chip microfluidic platforms, are given by Shawn O. Meade *et al*. in Chapter 10, *Microfluidic Flow Cytometry*: *Advancements Toward Compact, Integrated Systems*.

In Chapter 11 by Xin-Hua Hu and Jun Q. Lu, *Label-Free Cell Classification with a Diffraction Imaging Flow Cytometer*, aiming at the accurate modeling of light scattering from biological cells with realistic cell structures and the development of a high contrast diffraction imaging flow cytometer for experimental study, the authors are focused on the application of the FDTD method for modeling of coherent light scattering from cells. Numerical and experimental results are

presented and their implications to future improvement of the flow cytometry are discussed.

In Chapter 12 by Rabindra Tirouvanziam *et al.*, *An Integrative Approach for Immune Monitoring of Human Health and Disease by Advanced Flow Cytometry Methods*, the authors show key steps to move past the current limitations and truly enable the use of advanced flow cytometry tools for human research, promoting simplified, low-cost, and better standardized methods for sample collection, highlighting the enormous opportunities for research on reagents available for advanced flow cytometry analysis of human samples, and novel insights into relations of human immunity with age, gender, ethnicity, environmental exposure, health conditions, and therapies.

R. Dasgupta and P.K. Gupta, in Chapter 13, *Optical Tweezers and Cytometry*, give a brief introduction to optical tweezers and an overview of their use in cytometric applications, including measurements of viscoelastic properties of cells, in particular RBC, and Raman spectroscopic studies at single cell level. A few examples illustrating the potential of this approach for cytometric applications are also presented.

Chapter 14 by Valery V. Tuchin *et al.*, *In vivo Image Flow Cytometry*, presents one of the novel approaches in flow cytometry – *in vivo* video imaging digital flow cytometry. The fundamentals and instrumentation of video imaging flow cytometry, as well as spatial and temporal resolution of the method, are discussed. Experimental animal models, data on imaging and detection of individual cells in lymph and blood flows, and cell velocity measurements in lymph and blood vessels are presented and discussed. Intravessel RBC deformability measurement, monitoring of intralymphatic cell aggregation, and many other cell interaction phenomena are demonstrated and quantified.

Perspectives of the technique for disease diagnostics and monitoring and cell flow response on drugs, pollutions, and toxins are shown.

Chapter 15 by Stephen P. Morgan and Ian M. Stockford, *Instrumentation for In Vivo Flow Cytometry: A Sickle Cell Anemia Case Study*, discusses label-free monitoring of the properties of circulating blood cells for the *in vivo* monitoring of sickle cell anemia. For discriminating sickled RBCs in a background of normal cells, absorption measurements associated with sickle cell lower oxygen saturation and polarization measurements to identify their polymerization ability via cell adhesion to the vascular walls and, thus, more slow flow, are used. Illumination methods overcoming surface reflections, such as orthogonal polarization spectral imaging, dark field epi-illumination, and sidestream dark field illumination, are analyzed. In humans blood cell imaging has been performed either on the lower lip or under the tongue where the superficial mucosal tissue above the microcirculation is thinner than at other sites on the body. All steps of clinical instrumentation design, starting from discussion of the clinical needs for the measurements, the illumination and detection requirements, image processing methods for correction of image distortions, and a Monte Carlo model of the image formation process, up to engineering of the clinical prototype and presentation of clinical results are highlighted by the authors.

Accounting for the clinical importance of detection and quantification of circulating tumor cells (CTCs) for cancer diagnosis, staging, and treatment, in Chapter 16, *Advances in Fluorescence-Based In Vivo Flow Cytometry for Cancer Applications*, Cherry Greiner and Irene Georgakoudi, review the principles and instrumentation designs of fluorescence-based *in vivo* flow cytometry

(IVFC) and present data on the *in vivo* quantification of CTCs. The confocal and multiphoton microscopic techniques and systems adapted for the detection of fluorescently labeled CTCs in blood vessels are described. The noninvasive nature of IVFC systems and their capability to provide sensitive, continuous and dynamic monitoring of CTCs in blood flow are proved.

Chapter 17, *In Vivo Photothermal and Photoacoustic Flow Cytometry* by Valery V. Tuchin *et al.*, is devoted to presentation of the prospective approaches of IVFC that use laser-induced photothermal (PT) and photoacoustic (PA) effects. The authors analyze in detail integrated IVFC techniques combining a few different methods, such as PT imaging conjugated with thermo-lens and PA imaging, transmittance digital microscopy, and phase-sensitive and fluorescence imaging. The unique capabilities of the PT/PAFC (photoacoustic flow cytometry) technique for IVFC are illustrated in many examples of *in vivo* and *ex vivo* studies within lymph and blood vessels of animal models. Data on cell velocity measurements, detection, and real-time monitoring of circulating blood and lymph cells, bacteria, CTCs, contrast agents, and nanoparticles, and on quantification of cell interactions are presented. Perspectives of PT/PAFC technique for early diagnostics of cancer are discussed.

Martin J. Leahy and Jim O'Doherty, in Chapter 18, *Optical Instrumentation for the Measurement of Blood Perfusion, Concentration, and Oxygenation in Living Microcirculation*, compare the operation of an established microcirculation imaging technique, such as laser Doppler perfusion imaging (LDPI), which, for example, has been shown to accurately assess burn depth, with laser speckle perfusion imaging (LSPI) and tissue viability imaging (TiVi) in human skin tissue using the occlusion and reactive hyperaemia response. On the basis of the