

Optical Diagnostics of Living Cells III
Volume 3921

Optical Diagnostics of Living Cells III

Daniel L. Farkas

Robert C. Leif

Chairs/Editors

24-25 January 2000

San Jose, California

Sponsored by

SPIE—The International Society for Optical Engineering

IBOS—International Biomedical Optics Society

Published by

SPIE—The International Society for Optical Engineering

Proceedings of SPIE

Volume 3921

SPIE is an international technical society dedicated to advancing engineering and scientific applications of optical, photonic, imaging, electronic, and optoelectronic technologies.



The papers appearing in this book compose the proceedings of the technical conference cited on the cover and title page of this volume. They reflect the authors' opinions and are published as presented, in the interests of timely dissemination. Their inclusion in this publication does not necessarily constitute endorsement by the editors or by SPIE. Papers were selected by the conference program committee to be presented in oral or poster format, and were subject to review by volume editors or program committees.

Please use the following format to cite material from this book:

Author(s), "Title of paper," in *Optical Diagnostics of Living Cells III*, Daniel L. Farkas, Robert C. Leif, Editors, Proceedings of SPIE Vol. 3921, page numbers (2000).

ISSN 1605-7422
ISBN 0-8194-3537-6

Published by
SPIE—The International Society for Optical Engineering
P.O. Box 10, Bellingham, Washington 98227-0010 USA
Telephone 360/676-3290 (Pacific Time) • Fax 360/647-1445

Copyright ©2000, The Society of Photo-Optical Instrumentation Engineers.

Copying of material in this book for internal or personal use, or for the internal or personal use of specific clients, beyond the fair use provisions granted by the U.S. Copyright Law is authorized by SPIE subject to payment of copying fees. The Transactional Reporting Service base fee for this volume is \$15.00 per article (or portion thereof), which should be paid directly to the Copyright Clearance Center (CCC), 222 Rosewood Drive, Danvers, MA 01923. Payment may also be made electronically through CCC Online at <http://www.directory.net/copyright/>. Other copying for republication, resale, advertising or promotion, or any form of systematic or multiple reproduction of any material in this book is prohibited except with permission in writing from the publisher. The CCC fee code is 1605-7422/00/\$15.00.

Printed in the United States of America.

Conference Committees

Part A Advanced Techniques in Analytical Cytology IV

Conference Chair

Robert C. Leif, Newport Instruments

Program Committee

Dario Cabib, Applied Spectral Imaging Ltd. (Israel)

James F. Leary, University of Texas Medical Branch at Galveston

Calum E. MacAulay, British Columbia Cancer Research Center (Canada)

Jeffrey H. Price, University of California/San Diego

Alexander P. Savitsky, Institute of Biochemistry (Russia)

John A. Steinkamp, Los Alamos National Laboratory

Session Chairs

Microscopy I

Calum E. MacAulay, British Columbia Cancer Agency (Canada)

Robert C. Leif, Newport Instruments

Microscopy II

Robert C. Leif, Newport Instruments

James F. Leary, University of Texas Medical Branch at Galveston

Flow

Robert C. Leif, Newport Instruments

Jeffrey H. Price, University of California/San Diego

Dyes and Detection

John A. Steinkamp, Los Alamos National Laboratory

Robert C. Leif, Newport Instruments

Late Breaking News Session

Calum E. MacAulay, British Columbia Cancer Agency (Canada)

Robert C. Leif, Newport Instruments

Part B Functional Imaging and Optical Manipulation of Living Cells and Tissues

Conference Chair

Daniel L. Farkas, Carnegie Mellon University and University of Pittsburgh

Program Committee

Michael Cable, Xenogen Corporation
Pamela Reilly-Contag, Xenogen Corporation
Stefan W. Hell, Max-Planck Institute for Biophysical Chemistry (Germany)
Philip E. Hockberger, Northwestern University
Joseph R. Lakowicz, University of Maryland/Baltimore School of Medicine
Vickie J. LaMorte, Beckman Laser Institute and Medical Clinic
Leslie M. Loew, University of Connecticut Health Center
Peter T. C. So, Massachusetts Institute of Technology
Bruce J. Tromberg, Beckman Laser Institute and Medical Clinic
Alan S. Waggoner, Carnegie Mellon University

Session Chairs

Micromanipulation and Nanotechniques
Vickie J. LaMorte, Beckman Laser Institute and Medical Clinic

Spectral and Lifetime Imaging
Ammasi Periasamy, University of Virginia

In-vivo Optical Imaging
Pamela Reilly-Contag, Xenogen Corporation

Multiphoton Microscopy
Peter T. C. So, Massachusetts Institute of Technology

Contents

ix *Conference Committees*

PART A *Advanced Techniques in Analytical Cytology*

5 *Introduction*

SESSION 1 MICROSCOPY I

- 6 **Improvements to quantitative microscopy through the use of digital micromirror devices [3921-01]**
A. L. P. Dlugan, C. E. MacAulay, P. M. Lane, British Columbia Cancer Agency (Canada)
- 12 **In-vivo confocal microscopy based on the Texas Instruments digital micromirror device [3921-02]**
E. L. Botvinick, F. Li, Univ. of California/San Diego; S. Cha, Univ. of California/San Diego and Kangwon National Univ. (Korea); D. A. Gough, Y. Fainman, J. H. Price, Univ. of California/San Diego
- 20 **Cell enumeration and characterization in microvolume laser scanning cytometry: a multicolor image-processing package [3921-03]**
S. M. Norton, J. Winkler, L. J. Dietz, SurroMed, Inc.
- 31 **Magnification-corrected optical image splitting for simultaneous multiplanar acquisition [3921-04]**
L. K. Nguyen, Univ. of California/San Diego; M. E. Bravo-Zanoguera, Univ. of California/San Diego and Univ. Autónoma de Baja California (Mexico); A. L. Kellner, J. H. Price, Univ. of California/San Diego
- 41 **Software framework for scanning cytometry [3921-05]**
E. A. Hunter, Q3DM, Inc. and Univ. of California/San Diego; W. S. Callaway III, Q3DM, Inc.; J. H. Price, Q3DM, Inc. and Univ. of California/San Diego

SESSION 2 MICROSCOPY II

- 54 **Cell nuclear features for classification from fluorescence images [3921-06]**
S. Heynen, Univ. of California/San Diego; E. A. Hunter, J. H. Price, Q3DM, Inc.
- 66 **Analysis of the 3D spatial organization of cells and subcellular structures in tissue [3921-07]**
D. W. Knowles, C. Ortiz de Solorzano, A. Jones, S. J. Lockett, Lawrence Berkeley National Lab.
- 74 **Quantitative architectural analysis of bronchial intraepithelial neoplasia [3921-47]**
M. Guillaud, C. E. MacAulay, J. C. LeRiche, C. Dawe, J. Korbelik, S. Lam, British Columbia Cancer Agency (Canada) and Univ. of British Columbia (Canada)

SESSION 3 FLOW

- 84 **Application of a new novel data-mining technique to cytometry data [3921-09]**
J. F. Leary, S. R. McLaughlin, L. M. Reece, Univ. of Texas Medical Branch at Galveston
- 90 **Flow cytometry systems for drug discovery and development [3921-10]**
J. T. Ransom, Axiom Biotechnologies, Inc.; B. S. Edwards, F. W. Kuckuck, Univ. of New Mexico Cancer Research and Treatment Ctr.; A. Okun, D. K. Mattox, Axiom Biotechnologies, Inc.; E. R. Prossnitz, L. A. Sklar, Univ. of New Mexico Cancer Research and Treatment Ctr.
- 101 **Fluorescent molecular rotor for the study of membrane fluidity in endothelial cells under fluid shear stress [3921-11]**
M. A. Haidekker, J. A. Frangos, Univ. of California/San Diego

SESSION 4 DYES AND DETECTION

- 114 **New portable time-resolved photometer for monitoring the calcium dynamics of osteoblasts under mechanical and zero-gravity stimulation [3921-13]**
J. Struckmeier, J. Tenbosch, E. Klopp, M. Born, M. R. Hofmann, D. W. Jones, Philipps Univ. of Marburg (Germany)
- 124 **Methods to increase the luminescence of lanthanide (III) macrocyclic complexes [3921-14]**
J. R. Quagliano, Los Alamos National Lab.; R. C. Leif, Newport Instruments; L. M. Vallarino, S. A. Williams, Virginia Commonwealth Univ.

POSTER SESSION

- 136 **Reconstruction of 3D radial distribution of interphase chromatin in lymphocyte nucleus for various diseases [3921-17]**
I. Patrickeyev, P. Frick, Institute of Continuous Media Mechanics (Russia); A. V. Zhukotsky, Institute of Physical and Chemical Medicine (Russia)
- 147 **Sensitivity of detection of bacteria with fluorescent and luminescent phenotypes using different instruments [3921-18]**
L. Y. Brovko, M. W. Griffiths, Univ. of Guelph (Canada)
- 157 **Multiparameter breast cancer cell image analysis for objective estimation of nuclear grade: comparison with light microscopic observational data [3921-19]**
J. Berzins, U. Sneiders, D. Plegere, Latvian Cancer Ctr.; T. Freivalds, Institute of Experimental and Clinical Medicine/Univ. of Latvia; R. Grigalinovica, P. Stradins Hospital (Latvia)
- 163 **Refractive properties of separate erythrocytes of Chernobyl clean-up workers at different pH [3921-20]**
G. Mazarevica, Univ. of Latvia; T. Freivalds, Institute of Experimental and Clinical Medicine/Univ. of Latvia; R. Bruvere, N. Gabruseva, Biomedical Research and Study Ctr./Univ. of Latvia; A. Leice, Outpatient Clinic Dziednueciba (Latvia); T. Zvagule, P. Stradins Hospital (Latvia)

PART B *Functional Imaging and Optical Manipulation of Living Cells and Tissues*

SESSION 5 MICROMANIPULATION AND NANOTECHNIQUES

- 176 **Magnetic tweezers microscope for cellular manipulation [3921-24]**
C. Y. Dong, H. Huang, Massachusetts Institute of Technology; J. D. B. Sutin, Univ. of Illinois/Urbana-Champaign; H. S. Kwon, G. Cragg, R. Gilbert, Massachusetts Institute of Technology; R. T. Lee, Brigham and Womens Hospital; E. Gratton, Univ. of Illinois/Urbana-Champaign; R. D. Kamm, D. A. Lauffenburger, P. T. C. So, Massachusetts Institute of Technology

- 186 **Miniature optical microscope without imaging lens [3921-26]**
Y. Wang, Jet Propulsion Lab.

- 190 **Electrical-field-directed cell migration during respiratory epithelium wound repair [3921-27]**
J.-M. Zahm, B. Raby, A.-L. Hérard, E. Puchelle, N. Bonnet, INSERM (France)

- 197 **Determination of spring constant of laser-trapped particle by self-mixing interferometry [3921-45]**
G. Lai, Z. Ding, M. Wang, T. Sato, T. Sakakibara, S. Shinohara, Shizuoka Univ. (Japan)

- 205 **Creation of a virtual cutaneous tissue bank [3921-50]**
W. A. LaFramboise, S. Shah, R. W. Hoy, D. Letbetter, P. Petrosko, R. F. Vennare, P. C. Johnson, TissueInformatics, Inc.

SESSION 6 SPECTRAL AND LIFETIME IMAGING

- 210 **Special interaction between ligands and DNA [3921-29]**
X. Wang, X. Jiang, L. Cai, Z. H. Lu, Southeast Univ. (China)

- 218 **Fluorescence spectral imaging of organelle interaction [3921-30]**
E. Kohen, J. G. Hirschberg, C. Kohen, Univ. of Miami; D. O. Schachtschabel, M. Monti, Philipps Univ. of Marburg (Germany); R. Stanikunaite, Univ. of Miami

- 232 **Illuminating cellular structure and function in the early secretory pathway by multispectral 3D imaging in living cells [3921-31]**
J. Rietdorf, D. J. Stephens, European Molecular Biology Lab. (Germany); A. Squire, Imperial Cancer Research Fund (UK); J. Simpson, European Molecular Biology Lab. (Germany); D. T. Shima, Imperial Cancer Research Fund (UK); J.-P. Paccaud, Univ. of Geneva School of Medicine (Switzerland); P. I. H. Bastiaens, Imperial Cancer Research Fund (UK); R. Pepperkok, European Molecular Biology Lab. (Germany)

- 242 **Fluorescence lifetime imaging of pH in cells: investigation of factors influencing the pH calibration lifetime [3921-32]**
R. M. Andersson, Astrid Lindgren Children's Hospital (Sweden); K. Carlsson, A. Liljeberg, Royal Institute of Technology (Sweden); H. Brismar, Astrid Lindgren Children's Hospital (Sweden)

- 249 **Two-photon fluorescence spectroscopy for identification of healthy and malignant biological tissues [3921-33]**
M. G. Xu, M. Gu, Swinburne Univ. of Technology (Australia); E. W. Thompson, E. D. Williams, St. Vincent's Institute of Medical Research/Univ. of Melbourne (Australia)

SESSION 7 IN-VIVO OPTICAL IMAGING

- 256 **External optical imaging of freely moving mice with green fluorescent protein-expressing metastatic tumors [3921-35]**
M. Yang, AntiCancer, Inc., Univ. of California/San Diego, and Yokohama City Univ. School of Medicine (Japan); E. Baranov, AntiCancer, Inc.; H. Shimada, Yokohama City Univ. School of Medicine (Japan); A. R. Moossa, Univ. of California/San Diego; R. M. Hoffman, AntiCancer, Inc. and Univ. of California/San Diego
- 260 **Direct observation of liposome uptake by leukocytes in vivo in skin blood vessels using intravital fluorescence microscopy [3921-34]**
J. M. Devoisselle, Univ. de Montpellier I (France); S. R. Mordon, INSERM (France); S. Begu, Univ. de Montpellier I (France); T. Desmettre, INSERM (France)
- 270 **High-resolution x-ray CT screening of mutant mouse models [3921-36]**
M. J. Paulus, S. S. Gleason, H. Sari-Sarraf, D. K. Johnson, C. J. Foltz, D. W. Austin, M. E. Easterly, E. J. Michaud, M. S. Dhar, P. R. Hunsicker, Oak Ridge National Lab.; J. W. Wall, M. Schell, Univ. of Tennessee/Knoxville Medical Ctr.
- 280 **Fluorescence lifetime imaging (FLIM): simulation and error analysis [3921-46]**
M. Elangovan, P. Kionga-Kamau, A. Periasamy, Univ. of Virginia

SESSION 8 MULTIPHOTON MICROSCOPY

- 290 **Two-photon fluorescence confocal microscopy with a passively Q-switched Nd:YAG microchip laser [3921-38]**
C. J. H. Brennan, P. T. C. So, Massachusetts Institute of Technology
- 299 **Two-photon excitation energy transfer microscopy [3921-40]**
A. Periasamy, Univ. of Virginia
- 305 **Real-time Ca ion wave imaging in living rat cardiac muscle cells by a confocal multiphoton microscope with a microlens-pinhole array scanner [3921-41]**
K. Fujita, T. Kaneko, O. Nakamura, Osaka Univ. (Japan); M. Oyamada, T. Takamatsu, Kyoto Prefectural Univ. of Medicine (Japan); S. Kawata, Osaka Univ. (Japan)
- 313 **Improvement of confocal spectral precision distance microscopy (SPDM) (Invited Paper) [3921-42]**
P. Edelmann, C. Cremer, Univ. of Heidelberg (Germany)
- 321 **Nanocalocalization measurements in spatially modulated illumination microscopy using two coherent illumination beams [3921-43]**
B. Schneider, B. Albrecht, P. Jaekle, D. Neofotistos, S. Söding, T. Jäger, C. Cremer, Univ. of Heidelberg (Germany)

331	Two-photon excitation laser scanning microscopy of rabbit nasal septal cartilage following Nd:YAG-laser-mediated stress relaxation [3921-44] C. C. Kim, Yale Univ. School of Medicine and Beckman Laser Institute and Medical Clinic; V. P. Wallace, M. L. Coleno, X. Dao, B. J. Tromberg, B. J. F. Wong, Beckman Laser Institute and Medical Clinic
341	<i>Addendum</i>
343	<i>Author Index</i>

Part A

**ADVANCED TECHNIQUES IN ANALYTICAL
CYTOLOGY**

SESSION 1

Microscopy I

Introduction

The Advanced Techniques in Analytical Cytology IV meeting has been recognized by the International Society for Analytical Cytology (ISAC) as an ISAC-affiliated meeting. This is further evidence that SPIE BiOS meetings are now the place to learn about new techniques in analytical cytology. Many papers relevant to analytical cytology are spread throughout this volume and will appear in other BiOS 2000 proceedings. The Advanced Techniques in Analytical Cytology IV meeting contained many interesting papers on diverse subjects concerning new instrumentation and software, including standards, dyes, reagents, and applications.

The use of digital micromirror devices is a significant advance in microscopy, which includes, but is not limited to, making confocal microscopes with ultraviolet light excitation available at affordable prices. Other interesting microscope technology improvements were also described. Software advances to improve the classification and segmentation of nuclei were presented. The use of flow cytometry for drug discovery and development is a new and very interesting application. Dedicated instrumentation for achieving the discrimination of flow cytometry without the use of complex instrumentation can significantly expand the use of clinical measurements, such as CD4 quantitation in HIV patients. The dye studies included measurements of membrane fluidity and the use of lanthanide macrocyclic complexes without the need for time-gated instrumentation.

I have deliberately avoided mentioning any author by name because I believe that it would be inappropriate for me to steer the readers to any particular paper(s). I believe that all of the papers are informative and useful, and that each of the individual readers including myself, quite properly, has his or own interests.

I again wish to thank my cochairs, the other authors, the audience, and most of all you, the readers, for the success of our meetings.

Robert C. Leif

Improvements to Quantitative Microscopy Through the Use of Digital Micromirror Devices

Andrew L. P. Dlugan, Calum E. MacAulay, Pierre M. Lane

British Columbia Cancer Agency, 601 West 10th Avenue, Vancouver, BC, Canada, V5Z 1L3

ABSTRACT

All of the different modes of microscopy deliver light in a controlled fashion to the object to be examined and collect as much of the light containing the desired information about the object as possible.

The system being presented replaces the simple circular or annular diaphragms of a conventional microscope with digital micromirror devices (DMDs, made by Texas Instruments) to enable digital light microscopy. The DMDs are placed in the optical path at positions corresponding to the field and aperture diaphragms of a conventional microscope. This allows for more precise and flexible control over the spatial location, amount, and angles of the illumination light, and the light to be collected.

Digital light microscopy enables the improvement of existing modes of microscopy, specifically for quantitative microscopy applications. Confocal microscopy has been performed, realizing improvements in resolution, flexibility, and cost. Three different combinations of image acquisition and post-processing algorithms have been used to generate confocal images, as well as a tomographic reconstruction image.

Keywords: confocal microscopy, quantitative microscopy, digital micromirror device, digital light microscopy

1. INTRODUCTION

Microscopy is used to produce magnified representations of dynamic and stationary objects. There are many different modes of microscopy such as brightfield microscopy, darkfield microscopy, phase contrast microscopy, fluorescence microscopy, reflected light microscopy, confocal microscopy, etc. All of these deliver illumination light in a controlled fashion to the object to be examined and collect as much of the information-bearing light from the object as possible. Normally this is accomplished using either Köhler illumination for transmission microscopy or Köhler epi-illumination for fluorescence microscopy. Both of these methods use appropriately placed diaphragms and lenses to control both aperture (illumination cone) and size of the illuminated area of the sample. In particular, for Köhler illumination, these diaphragms are placed in: 1) the conjugate image plane of the sample (to control the size and shape of the illumination spot) as a field diaphragm or pinhole, and in 2) a conjugate image plane of the light source (to control the angles of the light illuminating the sample) for the aperture diaphragm. Usually, these diaphragms are simple irises (as in brightfield, epi-illumination, and fluorescence microscopy), but sometimes they are more complex, consisting of cutout rings of different diameters (as in darkfield microscopy).

1.1. Conjugate image planes

The field diaphragm determines the area of the sample that is illuminated. This diaphragm is placed at a conjugate plane of the sample as projected through the condenser or objective (epi-illumination). The primary image plane of the microscope is an equivalent conjugate plane as projected through the objective lens. Thus, a location which transmits light in the field diaphragm plane illuminates a minimized (as demagnified by the lens set) spot in the sample. Likewise, each location in the primary image plane corresponds to a minimized spot in the sample. These three planes form the first key set of conjugate image planes. If only a single location is illuminated and detected, and if these spots in the sample are in the exact same location, then one has a confocal microscope. In fact, a confocal microscope usually has a single pinhole detector and a point source illumination spot.

The second key set of conjugate image planes includes the light source and the aperture diaphragm planes on the illumination and detection sides of the sample. Each spot on the light source corresponds to a spot in the aperture diaphragm planes. For

a microscope using Köhler illumination, each spot in these planes also corresponds to an angle of light passing through the sample. Thus, in the illumination pathway of a bright field microscope, the aperture diaphragm controls the angles of light used to illuminate the sample. Likewise, on the image detection side of the microscope, the aperture diaphragm limits the angles of the light collected to form the image of the sample. For brightfield microscopy, these aperture diaphragms are simple fixed or adjustable circular irises. In darkfield microscopy, the illumination aperture will consist of a circular annulus limiting the illumination to a limited range of angles, while the detection aperture will usually accept a large range of detection light angles, but specifically not the angles used to illuminate the sample.

1.2. Microscopy using digital micromirror devices

The essence of the ideas being presented here is to replace these simple mechanical diaphragms with digital light processing units (digital micromirror devices¹, DMDs, made by Texas Instruments) or some other high contrast spatial light modulator. Placing a DMD at these locations allows for more sophisticated and precise control of the delivery of light to the sample and collection of light from the sample^{2,3}.

One of the most straightforward applications of using a DMD to perform dynamic illumination control is the creation of a confocal microscope^{4,5}. Illumination light is conducted from the light source, through an excitation light selection filter, reflected off a dichroic mirror, through a projection lens, onto a simple mirror, then onto the DMD. If one of the DMD micromirrors is ON, the light incident on it is reflected to the object lens. If the mirror is OFF, the light is conducted to a beam stop. The light conducted to the objective lens is transmitted and focused into a single location in the sample. Then, light generated by the sample (fluorescence) is collected by the objective lens, and transmitted to the primary image plane of the objective where the DMD is located. The DMD then reflects the light back to the simple mirror into the projection lens, through the dichroic mirror and an emission selection filter onto a light sensor that converts the light into an electronic signal for measurement. Each pixel in the primary image plane is mapped by the objective into a specific location in the sample. If the signal from the pixel which is mapped to the same location in the sample which is illuminated by the DMD, then information about that location in the sample is collected in exactly the same fashion as a regular confocal microscope.

1.3. Principal advantages of DMDs over mechanical diaphragms in microscopy

The advantages of DMDs over mechanical diaphragms are significant, particularly in quantitative microscopy: Each micromirror is individually controlled through software. This allows one to reproduce illumination patterns precisely. The switching time of the micromirrors is under 20 μ s. The fast switching time allows one to switch quickly between illumination patterns, and even entire modes of microscopy. Since there are no mechanical components to slide or rotate into the optical path, the possibility of disrupting the optical apparatus is eliminated. With diaphragms or annuli, one is limited to a small number of illumination patterns. The DMD can generate illumination patterns of essentially any shape and size to better match the sample being studied. The micromirrors can simulate 256 grey levels by switching ON and OFF many times within a single video frame.

2. RESULTS FROM DIGITAL LIGHT MICROSCOPY

Several image acquisition modes for digital light microscopy have been investigated. Semiconductor “targets” have been imaged under epi-illumination conditions. Biological samples have been imaged with transmission microscopy and fluorescence microscopy. Further, images have been collected using a single DMD in the field diaphragm position to control illumination pattern and size, and also with a DMD in the aperture diaphragm position to control illumination angles.

2.1. DMD placed in the field diaphragm plane

2.1.1. Demonstration of confocal condition

To demonstrate that digital light microscopy can be used to produce confocal images, a DMD was placed in the field diaphragm plane of a conventional microscope configured for epi-illumination. A plane mirror was used as the “sample”. By turning a single DMD mirror ON, one spot on the plane mirror was illuminated. The mirror was scanned in the z-direction along the optical axis. At each position, an image of the illumination spot was collected.