PROCEEDINGS OF SPIE

SPIDigitalLibrary.org/conference-proceedings-of-spie

Front Matter: Volume 8588

, "Front Matter: Volume 8588," Proc. SPIE 8588, Multiphoton Microscopy in the Biomedical Sciences XIII, 858836 (28 March 2013); doi: 10.1117/12.2021921

SPIE.

Event: SPIE BiOS, 2013, San Francisco, California, United States

Multiphoton Microscopy in the Biomedical Sciences XIII

Ammasi Periasamy

Karsten König

Peter T. C. So

Editors

3–5 February 2013

San Francisco, California, United States

Sponsored

SPIE

Cosponsored by

Becker and Hickel GmbH (Germany)

Boston Electronics Corporation (United States)

Coherent, Inc.

Intelligent Software Solutions, Inc. (United States)

JenLab GmbH (Germany)

Newport-Spectra Physics

Omega Optical, Inc. (United States)

Princeton Instruments

Semrock, Inc. (United States)

Carl Zeiss

Published by

SPIE

Volume 8588

Proceedings of SPIE, 1605-7422, V. 8588

SPIE is an international society advancing an interdisciplinary approach to the science and application of light.

Multiphoton Microscopy in the Biomedical Sciences XIII, edited by Ammasi Periasamy, Karsten König, Peter T. C. So,
Proc. of SPIE Vol. 8588, 858836 · © 2013 SPIE · CCC code: 1605-7422/13/\$18 · doi: 10.1117/12.2021921

Proc. of SPIE Vol. 8588 858836-1

The papers included in this volume were part of the technical conference cited on the cover and title page. Papers were selected and subject to review by the editors and conference program committee. Some conference presentations may not be available for publication. The papers published in these proceedings reflect the work and thoughts of the authors and are published herein as submitted. The publisher is not responsible for the validity of the information or for any outcomes resulting from reliance thereon.

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

Author(s), "Title of Paper," in *Multiphoton Microscopy in the Biomedical Sciences XIII*, edited by Ammasi Periasamy, Karsten König, Peter T. C. So, Proceedings of SPIE Vol. 8588 (SPIE, Bellingham, WA, 2013) Article CID Number.

ISSN: 1605-7422

ISBN: 9780819493576

Published by

SPIE

P.O. Box 10, Bellingham, Washington 98227-0010 USA

Telephone +1 360 676 3290 (Pacific Time) · Fax +1 360 647 1445

SPIE.org

Copyright © 2013, 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 \$18.00 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 copyright.com. 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 1605-7422/13/\$18.00.

Printed in the United States of America.

Publication of record for individual papers is online in the SPIE Digital Library.

SPIDigitalLibrary.org

Paper Numbering: Proceedings of SPIE follow an e-First publication model, with papers published first online and then in print and on CD-ROM. Papers are published as they are submitted and meet publication criteria. A unique, consistent, permanent citation identifier (CID) number is assigned to each article at the time of the first publication. Utilization of CIDs allows articles to be fully citable as soon as they are published online, and connects the same identifier to all online, print, and electronic versions of the publication. SPIE uses a six-digit CID article numbering system in which:

- The first four digits correspond to the SPIE volume number.
- The last two digits indicate publication order within the volume using a Base 36 numbering system employing both numerals and letters. These two-number sets start with 00, 01, 02, 03, 04, 05, 06, 07, 08, 09, 0A, 0B ... 0Z, followed by 10-1Z, 20-2Z, etc.

The CID Number appears on each page of the manuscript. The complete citation is used on the first page, and an abbreviated version on subsequent pages. Numbers in the index correspond to the last two digits of the six-digit CID Number.

Contents

xi	Conference Committee
xiii	Introduction
xv	In Memoriam: Prof. Robert M. Clegg (1945-2012)

KEYNOTE SESSION

8588 01	Enhanced resolution and sensitivity in fluorescence fluctuation measurements using multi-modal data acquisition and global analysis (Keynote Paper) [8588-1] K. M. Berland, N. R. Anthony, Emory Univ. (United States)
8588 02	Promising new wavelengths for multi-photon microscopy: thinking outside the Ti:Sapphire box (Keynote Paper) [8588-2] G. Norris, R. Amor, J. Dempster, Univ. of Strathclyde (United Kingdom); W. B. Amos, MRC Lab. of Molecular Biology (United Kingdom); G. McConnell, Univ. of Strathclyde (United Kingdom)

SESSION 1 CRS TECHNOLOGY

8588 06	Label-free observation of tissues by high-speed stimulated Raman spectral microscopy and independent component analysis (Invited Paper) [8588-6] Y. Ozeki, Osaka Univ. (Japan) and PRESTO, Japan Science and Technology Agency (Japan); Y. Otsuka, S. Sato, H. Hashimoto, Canon Inc. (Japan); W. Umemura, K. Sumimura, Osaka Univ. (Japan); N. Nishizawa, Nagoya Univ. (Japan); K. Fukui, K. Itoh, Osaka Univ. (Japan)
8588 08	Neuronal cell growth on polymeric scaffolds studied by CARS microscopy (Invited Paper) [8588-8] A. Enejder, H. Hagman, J. Kiskis, Chalmers Univ. of Technology (Sweden)
8588 09	Polarization-coded coherent anti-Stokes Raman scattering microscopy for nonresonant background suppression [8588-9] J. Lin, P. K. Upputuri, G. Li, H. Wang, Z. Huang, National Univ. of Singapore (Singapore)

SESSION 2 LASER SOURCES FOR CRS MICROSCOPY

8588 0B	Spectroscopic SRS imaging with a time-lens source synchronized to a femtosecond pulse shaper [8588-11] K. Wang, Cornell Univ. (United States); D. Zhang, K. Charan, M. N. Slipchenko, P. Wang, J.-X. Cheng, Purdue Univ. (United States); C. Xu, Cornell Univ. (United States)
---------	--

- 8588 0C **Development of CARS spectrometer using dual-wavelength electronically tuned laser** [8588-12]
Y. Maeda, Y. Nishimoto, H. Sato, Kwansei Gakuin Univ. (Japan)
- 8588 0E **Broadly tunable high-energy spectrally focused CARS microscopy with chemical specificity and high resolution for biological samples** [8588-14]
C. Brideau, K. Poon, P. K. Stys, Univ. of Calgary (Canada)
- 8588 0F **Fiber bundle based probe with polarization for coherent anti-Stokes Raman scattering microendoscopy imaging** [8588-15]
Z. Liu, Beijing Institute of Technology (China) and The Methodist Hospital Research Institute, Weill Cornell Medical College (United States); Z. Wang, X. Wang, X. Xu, X. Chen, J. Cheng, X. Li, The Methodist Hospital Research Institute, Weill Cornell Medical College (United States); S. Chen, J. Xin, Beijing Institute of Technology (China); S. T. Wong, The Methodist Hospital Research Institute, Weill Cornell Medical College (United States)

SESSION 3 APPLICATIONS OF CARS MICROSCOPY

- 8588 0I **Imaging plant tissues with coherent Raman scattering microscopy** [8588-19]
J. Moger, J. C. Mansfield, Univ. of Exeter (United Kingdom)
- 8588 0N **Combining multiphoton and CARS microscopy for skin imaging** [8588-24]
H. G. Breunig, JenLab GmbH (Germany) and Saarland Univ. (Germany); M. Weinigel, M. Kellner-Höfer, R. Bückle, JenLab GmbH (Germany); M. E. Darvin, J. Lademann, Charité Universitätsmedizin Berlin (Germany); K. König, JenLab GmbH (Germany) and Saarland Univ. (Germany)
- 8588 0O **Hyperspectral coherent anti-Stokes Raman scattering microscopy for in situ analysis of solid-state crystal polymorphs** [8588-25]
E. T. Garbaci, A. L. Fussell, Univ. Twente (Netherlands); S. Güres, Heinrich-Heine-Univ. Düsseldorf (Germany); J. P. Korterik, C. Otto, J. L. Herek, H. L. Offerhaus, Univ. Twente (Netherlands)
- 8588 0P **Raman microspectroscopy for visualization of peripheral nerves** [8588-26]
T. Minamikawa, Y. Harada, N. Koizumi, T. Takamatsu, Graduate School of Medical Science, Kyoto Prefectural Univ. of Medicine (Japan)

SESSION 4 FRET/FLIM/FCS MICROSCOPY-I

- 8588 0Q **Monitoring subunit rotation in single FRET-labeled F_0F_1 -ATP synthase in an anti-Brownian electrokinetic trap (Invited Paper)** [8588-27]
T. Heitkamp, H. Sielaff, A. Korn, M. Renz, Jena Univ. Hospital, Friedrich-Schiller-Univ. Jena (Germany); N. Zarrabi, Univ. of Stuttgart (Germany); M. Börsch, Jena Univ. Hospital, Friedrich-Schiller-Univ. Jena (Germany) and Univ. of Stuttgart (Germany)
- 8588 0R **Fluorescence lifetime imaging with near-infrared dyes (Invited Paper)** [8588-28]
W. Becker, V. Shcheslavskiy, Becker & Hickl GmbH (Germany)

- 8588 OS **Two photon fluorescence imaging of lipid membrane domains and potentials using advanced fluorescent probes** [8588-29]
V. Kilin, Z. Darwich, L. Richert, P. Didier, A. Klymchenko, Y. Mély, Biophotonics and Pharmacology Lab., CNRS, Faculté de Pharmacie (France)

SESSION 5 FRET/FLIM/FCS MICROSCOPY-II

- 8588 OU **Cell metabolism, tumour diagnosis and multispectral FLIM (Invited Paper)** [8588-31]
A. Rück, C. Hauser, S. Lorenz, S. Mosch, S. Rotte, M. Kessler, S. Kalinina, Institute for Laser Technologies in Medicine and Metrology, Univ. Ulm (Germany)
- 8588 OV **Time-resolved spectroscopy of endogenous NAD(P)H in *Gluconobacter oxydans* (Invited Paper)** [8588-32]
J. Horilova, International Laser Ctr. (Slovakia) and Pavol Jozef Safarik Univ. (Slovakia); K. Kromkova, International Laser Ctr. (Slovakia) and Univ. Komenského v Bratislave (Slovakia); M. Bucko, A. Illesova, A. Vikartovska, Institute of Chemistry (Slovakia); V. Stefuca, Axxence Slovakia s.r.o. (Slovakia); A. Mateasik, D. Chorvat Jr., A. Chorvatova, International Laser Ctr. (Slovakia)
- 8588 OX **Development of a fast TCSPC FLIM-FRET imaging system** [8588-34]
S. P. Poland, S. Coelho, King's College London (United Kingdom); N. Krstajić, D. Tyndall, R. Walker, The Univ. of Edinburgh (United Kingdom); J. Monypenny, King's College London (United Kingdom); D. D.-U. Li, Univ. of Sussex (United Kingdom); R. Henderson, The Univ. of Edinburgh (United Kingdom); S. Ameer-Beg, King's College London (United Kingdom)
- 8588 OY **A fiber-laser-based stimulated Raman scattering spectral microscope** [8588-35]
K. Nose, Osaka Univ. (Japan); Y. Ozeki, Osaka Univ. (Japan) and PRESTO, Japan Science and Technology Agency (Japan); T. Kishi, K. Sumimura, Y. Kanematsu, K. Itoh, Osaka Univ. (Japan)

SESSION 6 TECHNOLOGY DEVELOPMENT-I

- 8588 10 **Ultra-deep imaging of turbid samples by enhanced photon harvesting (Invited Paper)** [8588-37]
V. Crosignani, A. Dvornikov, E. Gratton, Univ. of California, Irvine (United States)
- 8588 12 **Clinical studies of pigmented lesions in human skin by using a multiphoton tomograph (Invited Paper)** [8588-39]
M. Balu, Beckman Laser Institute and Medical Clinic (United States); K. M. Kelly, C. B. Zachary, R. M. Harris, Univ. of California, Irvine (United States); T. B. Krasieva, Beckman Laser Institute and Medical Clinic (United States); K. König, JenLab GmbH (Germany) and Saarland Univ. (Germany); B. J. Tromberg, Beckman Laser Institute and Medical Clinic (United States)
- 8588 13 **Optical clearing and multiphoton imaging of paraffin-embedded specimens** [8588-40]
J. W. Wilson, S. Degan, M. C. Fischer, W. S. Warren, Duke Univ. (United States)

SESSION 7 TECHNOLOGY DEVELOPMENT-II

- 8588 14 **High contrast in vivo bioimaging using multiphoton upconversion in novel rare-earth-doped fluoride upconversion nanoparticles (Invited Paper)** [8588-41]
G. Chen, Univ. at Buffalo, SUNY (United States) and Harbin Institute of Technology (China); C. Yang, Harbin Institute of Technology (China); P. N. Prasad, Univ. at Buffalo, SUNY (United States)
- 8588 17 **Multifocal multiphoton microscopy with adaptive optical correction** [8588-44]
S. Coelho, S. Poland, King's College London (United Kingdom); N. Krstajic, The Univ. of Edinburgh (United Kingdom); D. Li, Univ. of Sussex (United Kingdom); J. Monypenny, King's College London (United Kingdom); R. Walker, D. Tyndall, The Univ. of Edinburgh (United Kingdom); T. Ng, King's College London (United Kingdom); R. Henderson, The Univ. of Edinburgh (United Kingdom); S. Ameer-Beg, King's College London (United Kingdom)
- 8588 19 **Multiphoton cryo microscope with sample temperature control** [8588-46]
H. G. Breunig, JenLab GmbH (Germany) and Saarland Univ. (Germany); A. Uchugonova, JenLab GmbH (Germany); K. König, JenLab GmbH (Germany) and Saarland Univ. (Germany)
- 8588 1A **In vivo reactive neural plasticity investigation by means of correlative two photon: electron microscopy (Invited Paper)** [8588-47]
A. L. Allegra Mascaro, Univ. of Florence (Italy); P. Cesare, Fondazione Santa Lucia (Italy) and Univ. of Turin (Italy); L. Sacconi, Univ. of Florence (Italy) and National Institute of Optics-National Research Council (Italy); G. Grasselli, G. Mandolesi, Fondazione Santa Lucia (Italy); B. Maco, G. Knott, Ecole Polytechnique Fédérale de Lausanne (Switzerland); L. Huang, V. De Paola, Imperial College London (United Kingdom); P. Strata, Fondazione Santa Lucia (Italy) and Univ. of Turin (Italy); F. S. Pavone, Univ. of Florence (Italy)

SESSION 8 TECHNOLOGY DEVELOPMENT-III

- 8588 1B **Probing the spatiotemporal relationship between intracellular Ca²⁺ release and action potential propagation in cardiomyocytes by ultrafast multi-photon random access microscopy (Invited Paper)** [8588-48]
L. Sacconi, INO-National Research Council (Italy) and European Lab. for Non-linear Spectroscopy (Italy); C. Crocini, European Lab. for Non-linear Spectroscopy (Italy); R. Coppini, C. Ferrantini, C. Tesi, Univ. of Florence (Italy); P. Yan, L. Loew, Univ. of Connecticut Health Ctr. (United States); E. Cerbai, C. Poggesi, Univ. of Florence (Italy); F. S. Pavone, INO-National Research Council (Italy) and European Lab. for Non-linear Spectroscopy (Italy) and Univ. of Florence (Italy)
- 8588 1E **New developments in clinical CARS** [8588-52]
M. Weinigel, JenLab GmbH (Germany); H. G. Breunig, JenLab GmbH (Germany) and Saarland Univ. (Germany); M. Kellner-Höfer, R. Bückle, JenLab GmbH (Germany); M. Darvin, J. Lademann, Charité Universitätsmedizin Berlin (Germany); K. König, JenLab GmbH (Germany) and Saarland Univ. (Germany)

- 8588 1I **Photon absorption in step-wise multi-photon activation fluorescence (SMPAF) of Sepia melanin** [8588-56]
Z. Lai, J. Kerimo, C. DiMarzio, Northeastern Univ. (United States) and Bernard M. Gordon Ctr. for Subsurface Sensing and Imaging System (United States)
- 8588 1J **Multimodal nonlinear optical microscopy used to discriminate human colon cancer** [8588-57]
J. Adur, Univ. Estadual de Campinas (Brazil) and Univ. Nacional de Entre Ríos (Argentina); V. B. Pelegati, Univ. Estadual de Campinas (Brazil); M. Bianchi, Univ. Nacional de Entre Ríos (Argentina); A. A. de Thomaz, M. O. Baratti, H. F. Carvalho, Univ. Estadual de Campinas (Brazil); V. H. Casco, Univ. Nacional de Entre Ríos (Argentina); C. L. Cesar, Univ. Estadual de Campinas (Brazil)
- 8588 1L **Two-photon excited endogenous fluorescence for label-free in vivo imaging ingestion of disease-causing bacteria by human leukocytes** [8588-110]
Y. Zeng, B. Yan, Q. Sun, S. K. Teh, W. Zhang, Z. Wen, J. Y. Qu, Hong Kong Univ. of Science and Technology (Hong Kong, China)

SESSION 9 SHG/THG MICROSCOPY-I

- 8588 1N **The arrangement of fibrous collagen in cornea using second harmonic generation (SHG) microscopy (Invited Paper)** [8588-61]
Y. Mega, J. McLean, R. Zareian, S. Karasek, Z. Lai, C. DiMarzio, Northeastern Univ. (United States)
- 8588 1O **Hierarchical model of fibrillar collagen distribution for polarization-resolved SHG microscopy** [8588-62]
A. E. Tuer, Univ. of Toronto (Canada); M. K. Akens, Sunnybrook Health Sciences Ctr. (Canada); S. Krouglov, D. Sandkuijl, Univ. of Toronto (Canada); B. C. Wilson, Ontario Cancer Institute (Canada); C. M. Whyne, Sunnybrook Health Sciences Ctr. (Canada); V. Barzda, Univ. of Toronto (Canada)
- 8588 1Q **Multiphoton microscopy based cryo-imaging of inflated frozen human lung sections at -60°C in healthy and COPD lungs** [8588-64]
T. Abraham, Penn State College of Medicine (United States); D. Kayra, A. Zhang, The James Hogg Research Ctr., Univ. of British Columbia (Canada); M. Suzuki, Hokkaido Univ. School of Medicine (Canada); J. McDonough, W. M. Elliott, The James Hogg Research Ctr., Univ. of British Columbia (Canada); J. D. Cooper, Univ. of Pennsylvania (United States); J. C. Hogg, The James Hogg Research Ctr., Univ. of British Columbia (Canada)

SESSION 10 SHG/THG MICROSCOPY-II

- 8588 1R **Imaging leukocytes in vivo with third harmonic generation microscopy** [8588-65]
C.-K. Tsai, C.-K. Chen, Y.-S. Chen, P.-C. Wu, T.-Y. Hsieh, H.-W. Liu, C.-Y. Yeh, W.-L. Lin, J.-S. Chia, T.-M. Liu, National Taiwan Univ. (Taiwan)

- 8588 1U **Nonlinear optical microscopy and microspectroscopy of oral precancers and early cancer** [8588-68]
G. Vargas, Univ. of Texas Medical Branch (United States); K. Edward, The Univ. of the West Indies (Jamaica)
- 8588 1W **Chirality study inside biological tissue by second harmonic generation circular dichroism** [8588-70]
K.-J. Hsu, H. Lee, G.-Y. Zhuo, P.-H. Chao, S.-W. Chu, National Taiwan Univ. (Taiwan)
- 8588 1X **Adaptive nonlinear microscopy for whole tissue imaging** [8588-71]
M. C. Müllenbroich, Univ. of Strathclyde (United Kingdom); E. J. McGhee, The Beatson Institute for Cancer Research (United Kingdom); A. J. Wright, Univ. of Nottingham (United Kingdom); K. I. Anderson, The Beatson Institute for Cancer Research (United Kingdom); K. Mathieson, Univ. of Strathclyde (United Kingdom)
- 8588 1Y **3D quantitative Fourier analysis of second harmonic generation microscopy images of collagen structure in cartilage** [8588-72]
E. I. Romijn, M. B. Lilledahl, Norwegian Univ. of Science and Technology (Norway)

POSTER SESSION

- 8588 1Z **Chemical-contrast imaging with pulse-shaping based pump-probe spectroscopy (JenLab Young Investigator Award Contender)** [8588-59]
D. C. Flynn, A. R. Bhagwat, J. P. Ogilvie, Univ. of Michigan (United States)
- 8588 20 **Optical metabolic imaging of live tissue cultures (JenLab Young Investigator Award)** [8588-73]
A. J. Walsh, R. S. Cook, Vanderbilt Univ. (United States); C. L. Arteaga, Vanderbilt Univ. (United States) and Vanderbilt Univ. Ingram Cancer Ctr. (United States); M. C. Skala, Vanderbilt Univ. (United States)
- 8588 21 **Stimulated Raman microscopy without ultrafast lasers (JenLab Young Investigator Award Contender)** [8588-74]
Z. Meng, G. I. Petrov, V. V. Yakovlev, Texas A&M Univ. (United States)
- 8588 22 **Multiphoton imaging to distinguish grana and starch inside an intact leaf (JenLab Young Investigator Award Contender)** [8588-75]
M.-Y. Chen, G.-Y. Zhuo, P.-F. Chen, P.-C. Wu, T.-M. Liu, S.-W. Chu, National Taiwan Univ. (Taiwan)
- 8588 23 **Imaging molecular structure with Stokes-polarimeter based second harmonic generation microscopy (JenLab Young Investigator Award Contender)** [8588-76]
N. Mazumder, J. Qiu, C.-W. Hu, F.-J. Kao, National Yang-Ming Univ. (Taiwan)
- 8588 24 **Ultra-deep penetration of temporally-focused two-photon excitation (JenLab Young Investigator Award Contender)** [8588-77]
G. Sela, H. Dana, S. Shoham, Technion-Israel Institute of Technology (Israel)

- 8588 25 **Maximum imaging depth comparison in porcine vocal folds using 776-nm vs. 1552-nm excitation wavelengths (JenLab Young Investigator Award Contender)** [8588-78]
M. Yildirim, O. Ferhanoglu, The Univ. of Texas at Austin (United States); J. B. Kobler, S. M. Zeitel, Massachusetts General Hospital (United States); A. Ben-Yakar, The Univ. of Texas at Austin (United States)
- 8588 26 **Two-photon-based structured illumination microscopy applied for superresolution optical biopsy** [8588-79]
C. H. Yeh, S. Y. Chen, National Central Univ. (Taiwan)
- 8588 2D **Evaluation of the oxidative stress of psoriatic fibroblasts based on spectral two-photon fluorescence lifetime imaging** [8588-86]
D. Kapsokalyvas, V. Barygina, Univ. of Florence (Italy); R. Cicchi, Univ. of Florence (Italy) and INO-National Research Council (Italy); C. Fiorillo, F. S. Pavone, Univ. of Florence (Italy)
- 8588 2E **Clinical multiphoton endoscopy with FLIM capability (Multiphoton Microscopy Best Poster Award)** [8588-87]
M. Weinigel, JenLab GmbH (Germany); H. G. Breunig, JenLab GmbH (Germany) and Saarland Univ. (Germany); P. Fischer, M. Kellner-Höfer, R. Bückle, JenLab GmbH (Germany); K. König, JenLab GmbH (Germany) and Saarland Univ. (Germany)
- 8588 2I **Multi-color femtosecond source for simultaneous excitation of multiple fluorescent proteins in two-photon fluorescence microscopy (Multiphoton Microscopy Best Poster Award)** [8588-91]
K. Wang, Cornell Univ. (United States); T.-M. Liu, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States) and National Taiwan Univ. (Taiwan); J. Wu, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States); N. G. Horton, Cornell Univ. (United States); C. P. Lin, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States); C. Xu, Cornell Univ. (United States)
- 8588 2J **Analysis of spectrally resolved autofluorescence images by support vector machines** [8588-92]
A. Mateasik, D. Chorvat, A. Chorvatova, International Laser Ctr. (Slovakia)
- 8588 2K **Two-photon fluorescence stereomicroscopy with Bessel beams** [8588-93]
Y. Yang, M. Lei, J. Zheng, R. Li, S. Yan, B. Yao, Xi'an Institute of Optics and Precision Mechanics (China); T. Ye, Xi'an Institute of Optics and Precision Mechanics (China) and The Univ. of Alabama at Birmingham (United States)
- 8588 2L **Two-photon microscopy for real-time monitoring of focused ultrasound-mediated drug delivery to the brain in a mouse model of Alzheimer's disease** [8588-94]
A. Burgess, N. Eterman, Sunnybrook Research Institute (Canada); I. Aubert, K. Hynynen, Sunnybrook Research Institute (Canada) and Univ. of Toronto (Canada)
- 8588 2M **Detection of calcium waves in mice heart tissue with multispot two-photon imaging** [8588-95]
C. de Mauro, C. A. Cecchetti, D. Alfieri, Light4Tech Firenze S.r.l. (Italy); G. Borile, A. Urbani, M. Mongillo, Venetian Institute of Molecular Medicine (Italy); F. S. Pavone, Univ. of Florence (Italy)

- 8588 2R **A study on the application of chirped photonic crystal fiber in multiphoton microscopy** [8588-100]
J. Yu, The Univ. of British Columbia (Canada); H. Zeng, H. Lui, The Univ. of British Columbia (Canada) and The BC Cancer Agency Research Ctr. (Canada) and Vancouver Coastal Health Research Institute (Canada); J. S. Skibina, Saratov State Univ. (Russian Federation); G. Steinmeyer, Max-Born-Institut für Nichtlineare Optik und Kurzzeitspektroskopie (Germany); S. Tang, The Univ. of British Columbia (Canada)
- 8588 2S **Corneal imaging and refractive index measurement using a combined multiphoton microscopy and optical coherence tomography system (Multiphoton Microscopy Best Poster Award)** [8588-101]
T. Lai, S. P. Chong, Y. Zhou, G. Moloney, S. Tang, The Univ. of British Columbia (Canada)
- 8588 2U **A preliminary investigation on the interaction between sol-gel immobilized glucose oxidase and freely diffusing glucose by means of two-photon microscopy** [8588-103]
I. Delfino, Univ. della Tuscia (Italy); M. Portaccio, M. De Rosa, M. Lepore, Seconda Univ. di Napoli (Italy)
- 8588 2W **In vivo imaging of dermal collagen in skin burn by collagen-sensitive second-harmonic-generation microscopy** [8588-106]
T. Yasui, Univ. of Tokushima (Japan) and Osaka Univ. (Japan); R. Tanaka, Osaka Univ. (Japan); E. Hase, Univ. of Tokushima (Japan); S. Fukushima, T. Araki, Osaka Univ. (Japan)
- 8588 2X **In vivo imaging of collagen fiber orientation with rapid polarization-resolved SHG microscopy (Multiphoton Microscopy Best Poster Award)** [8588-107]
Y. Tanaka, Osaka Univ. (Japan); E. Hase, Univ. of Tokushima (Japan); S. Fukushima, Osaka Univ. (Japan); T. Yasui, Osaka Univ. (Japan) and Univ. of Tokushima (Japan); T. Araki, Osaka Univ. (Japan)
- 8588 32 **Fluorescence lifetime imaging with pulsed diode laser enabled stimulated emission** [8588-114]
J. Ge, Y. Wang, C. Kuang, Zhejiang Univ. (China); S.-S. Lee, N. Mazumder, F.-J. Kao, National Yang-Ming Univ. (Taiwan)

Author Index

Conference Committee

Symposium Chairs

James Fujimoto, Massachusetts Institute of Technology
(United States)
R. Rox Anderson, Wellman Center for Photomedicine, Massachusetts
General Hospital (United States) and Harvard School of Medicine
(United States)

Program Track Chairs

Ammasi Periasamy, University of Virginia (United States)
Daniel L. Farkas, The University of Southern California (United States)

Conference Chairs

Ammasi Periasamy, University of Virginia (United States)
Karsten König, JenLab GmbH (Germany)
Peter T. C. So, Massachusetts Institute of Technology (United States)

Conference Program Committee

Wolfgang Becker, Becker & Hickl GmbH (Germany)
Alberto Diaspro, Istituto Italiano di Tecnologia (Italy)
Chen-Yuan Dong, National Taiwan University (Taiwan)
Kevin W. Eliceiri, University of Wisconsin-Madison (United States)
Scott E. Fraser, California Institute of Technology (United States)
Paul M. W. French, Imperial College London (United Kingdom)
Hans Gerritsen, Utrecht University (Netherlands)
Enrico Gratton, University of California, Irvine (United States)
Min Gu, Swinburne University of Technology (Australia)
Stefan W. Hell, Max-Planck-Institut für biophysikalische Chemie
(Germany)
Brian A. Herman, The University of Texas Health Science Center at San
Antonio (United States)
Satoshi Kawata, Osaka University (Japan)
Fu-Jen Kao, National Yang-Ming University (Taiwan)
Arnd K. Krueger, Spectra-Physics®, a Newport Corporation Brand
(United States)
Joseph R. Lakowicz, University of Maryland School of Medicine
(United States)
Steve M. McDonald, Coherent, Inc. (United States)
Angelika C. Rueck, Universität Ulm (Germany)
Junle Qu, Shenzhen University (China)

Steven S. Vogel, National Institutes of Health (United States)
Paul W. Wiseman, McGill University (Canada)
X. Sunney Xie, Harvard University (United States)
Bernhard Zimmermann, Carl Zeiss Jena GmbH (Germany)
Warren R. Zipfel, Cornell University (United States)

Session Chairs

Keynote Session

Ammasi Periasamy, University of Virginia (United States)

- 1 CRS Technology
Ji-Xin Cheng, Purdue University (United States)
- 2 Laser Sources for CRS Microscopy
Eric O. Potma, University of California, Irvine (United States)
- 3 Applications of CARS Microscopy
Annika M. Enejder, Chalmers University of Technology (Sweden)
- 4 FRET/FLIM/FCS Microscopy-I
Angelika C. Rueck, Universität Ulm (Germany)
- 5 FRET/FLIM/FCS Microscopy-II
Michael Börsch, Friedrich-Schiller-Universität Jena (Germany)
- 6 Technology Development-I
Peter T. C. So, Massachusetts Institute of Technology (United States)
- 7 Technology Development-II
Peter T. C. So, Massachusetts Institute of Technology (United States)
- 8 Technology Development-III
Karsten König, Universität des Saarlandes (Germany)
- 9 SHG/THG Microscopy-I
Francesco Saverio Pavone, European Laboratory for Non-linear Spectroscopy (Italy)
- 10 SHG/THG Microscopy-II
Paul J. Campagnola, University of Wisconsin-Madison (United States)

Poster Session

Aisada Uchugonova, Universität des Saarlandes (Germany)
Holly L. Aaron, University of California, Berkeley (United States)
Dusan Chorvat, International Laser Centre in Bratislava (Slovakia)
Kevin W. Eliceiri, University of Wisconsin-Madison (United States)

Introduction

Multiphoton microscopy has been established as the 3-D imaging method of choice for studying biomedical specimens from single cells to whole animals with sub-micron resolution. Two decades have passed since the realization of two-photon microscopy. The ever-expanding scope of applications and the continuing instrumental innovations require a forum where new ideas can be exchanged and presented. Our conference at the SPIE BIOS 2013 meeting continues to address this need.

This is the 13th year of this conference and we start our conference with three keynote lectures from leaders in the field: Drs. Keith Berland from Emory University, United States, Gail McConnell, University of Strathclyde, United Kingdom, and Xiaoliang Sunney Xie, Harvard University, United States.

For the second year in a row, the conference is extremely pleased to have the JenLab Young Investigator Award, in addition to our regular poster awards. This award is donated by Dr. Prof. Karsten König, President and Founder of JenLab GmbH, Germany. The award selection committee includes doctors Arnd Krueger (NewPort-Spectra Physics), Conor Evans (Harvard Univ.), Paul Campagnola (Univ. of Wisconsin) and the three conference chairs. The selection process includes the abstract, manuscript and poster presentation. Two finalists are selected for oral presentation after the poster presentation. The two finalists are (1) Mr. Murat Yildirim, The University of Texas at Austin [Paper 8588-78], and (2) Ms. Alex J. Walsh, Vanderbilt University [Paper 8588-73]. Vocal fold scarring is one of the major causes of voice disorders and may arise from overuse or post-surgical wound healing. Mr. Murat has compared a maximum imaging depth using two photon autofluorescence and second harmonic generation with third-harmonic generation imaging modalities for superior porcine vocal folds using his custom-built system. The other candidate, Ms. Alex Walsh, investigated the stability over time of two-photon auto-fluorescence imaging of NADH and FAD in live-cultured tissues. Her results demonstrated that cultured tissues remain viable for at least several days post excision. Furthermore, the optical redox ratio, NADH fluorescence lifetime, and FAD fluorescence lifetime do not significantly change in the cultured tissues over time.

Ms. Alex Walsh from Vanderbilt University was selected as the winner of the JenLab Young Investigator Award 2013. Please visit the SPIE URL to see the award winning pictures.

The four poster award winners are Ke Wang (Cornell University, United States), Martin Weinigel (JenLab GmbH), Tom Lai (The University of British Columbia, Canada), and Yuji Tanaka, (Osaka University, Japan).

This process above allows the presenters to provide a more in-depth discussion of their subject. Some of the most valuable contributions in this volume are articles written by highly experienced practitioners of multi-photon microscopy. They have enumerated the most important considerations in designing multi-photon microscopes and imaging

experiments. Further, updates on the state-of-the-art commercial multi-photon microscope systems are presented. This volume also includes articles describing some recent advances in major multi-photon microscope components and applications including laser light sources, ultra-fast optics, filters, FRET, FLIM, FCS, Raman, CARS, SRS and CRS microscopy and spectroscopy, single molecule, super-resolution imaging, endoscopy, and various scientific and clinical applications.

On a personal note, the conference chairs are grateful for the participation of all authors, and acknowledge the innovation-driven manufacturers (Becker & Hickl GmbH, Boston Electronics, Carl Zeiss, Chroma Technology, Coherent, ISS, JenLab GmbH, Newport-Spectra Physics, Princeton Instruments and Semrock) for their enthusiastic support in organizing this conference successfully for the last 13 years. We look forward to other exciting conferences in the second decade and welcome your continued participation and support.

**Ammasi Periasamy
Karsten König
Peter T. C. So**

In Memoriam

**Prof. Robert M. Clegg
(1945-2012)**

One personal note to the Fluorescence Microscopy Community... We are deeply sorry to have lost a great scientist in our field. Prof. Robert M. Clegg, University of Illinois at Urbana Champaign passed away on October 15, 2012 from complications arising from cancer. He is survived by his wife and three sons.

Prof. Clegg was born on July 18, 1945, in Providence, Rhode Island. He received his doctorate in physical chemistry in 1974 from Cornell University. Professor E. L. Elson supervised his dissertation entitled "Relaxation Kinetics Applying Repetitive Pressure Perturbations." Following graduation, Bob worked as a postdoctoral research associate in the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany. He was promoted to senior staff research associate in the Department of Molecular Biology where he developed state-of-the-art instruments to investigate the structure of nucleic acids, and to apply photo-physical approaches for clinical applications using FLIM and FRET techniques. Prof. Clegg accepted a position as Professor of Physics and Bioengineering at the University of Illinois at Urbana-Champaign in 1998, where he remained for the rest of his career.

Prof. Clegg was an avid student of the history of science with a special interest in FRET and FLIM. His colleagues considered him "a walking library of FLIM and FRET." Prof. Clegg was also a dedicated and beloved teacher, always searching for simple ways to convey complex biophysical ideas to his students. He was extremely generous with his time and intellect to the ultimate benefit of his students and colleagues. Prof. Clegg presented a number of papers at SPIE conferences and he was one of the Keynote Speakers at the Multiphoton Microscopy in the Biomedical Sciences IX conference, with a title "What is behind all those lifetimes anyway, and where do we go from here?" Prof. Clegg has been teaching (2003-2012) at the international annual workshop on FRET Microscopy organized at the W.M. Keck Center for Cellular Imaging at the University of Virginia, Charlottesville. The FRET and FLIM community will surely miss his absence in future scientific meetings.