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Introduction

Thirty-one years have passed since the realization of two-photon laser scanning microscopy. This conference has been running for the last 21 years. Due to the COVID19 pandemic, the conference become a Digital Forum.

Briefly, considering the historical perspective of multiphoton microscopy, in 1931, Maria Göppert-Mayer reported the quantum mechanical formulation of two-photon molecular excitation in her doctoral thesis[1]. Two-photon excited fluorescence was finally demonstrated by Kaiser and Garrett[2] shortly after the invention of the laser in 1960. Sheppard and co-workers developed the SHG microscope[3] for solid state specimens and Denk, Strickler, and Webb developed a non-linear fluorescence microscope and first showed that it can be applied to image biological system noninvasively[4]. Since then, the usage and the development of multiphoton microscopy has increased tremendously. More importantly, the commercialization of multiphoton imaging systems by Bio-Rad did increase awareness and application of this technology in biomedical imaging. It should also be recognized that laser companies (Spectra Physics and Coherent) played a key role in introducing tunable (700-1100 nm; currently from 680 to 1300 nm) femtosecond infrared-pulsed laser systems (Ti: sapphire) for multiphoton imaging. Moreover, the future will bring even easier-to-use equipment and increased sensitivity, which will allow greater flexibility in the simultaneous imaging of multiple fluorophores while images are collected over time and at greater depths inside tissue/live animals[5].

Multiphoton microscopy has been established as the 3-D imaging method of choice for studying living biomedical specimens from single cells and whole animals to patients with sub-micron resolution. 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 BIOS2021 Digital Forum continues to address this need.

This is the 21st year of this conference and we start with Keynote lectures from leaders in various fields in multiphoton microscopy development and applications. More importantly, we had invited speakers from around the world share their ideas and achievements using this novel technology.

(1) Alfred Vogel (Germany). Leaving any number of photons behind: Adventures in structural neurobiology
(2) Melissa Skala, University Wisconsin at Madison (United States), Evolution of multiphoton microscopy over three decades: Current perspectives and future directions

Due to the pandemic, we did not award the JenLab Young Investigators award and poster award.

On the exciting note, we had a great Networking Session via Zoom held on March 7th. The title of the Network session was **"New Trends in Multiphoton Microscopy".** Thanks to Ms. Dawn Jackson, SPIE, for arranging the Network session. Many investigators around the world participated in the Network session and gave an exciting talk in that session. About 67 participants from around the world participated in the Network session. This Network session was Chaired by the Conference Chair, Prof. Ammasi Periasamy, University of Virginia. Here is the list of Investigators who presented their interesting work.

1. Prof. Peter So, MIT, Boston (USA)- De-scattering with Excitation Patterning (DEEP) Enables Rapid Wide-field Imaging Through Scattering Media
2. Prof. Ji-Xin Cheng, Boston University, Boston (USA) - multiphoton microscopy using two laser beams
3. Prof. Michelle A. Digman, University of California at Irvine (USA) - 2p hyperspectral Imaging
4. Prof. Fu-Jen Kao, National Yang Ming University, Taipei (Taiwan) - Quantitative Pump-Probe Microscopy with Stimulated Emission
5. Prof. Junle QU, ShenZhen University (China) - nonlinear optical imaging for photobiomodulation of brain disease
6. Dr. Angelika Rueck, Ulm University (Germany) – New Trends in 2p Metabolic FLIM
7. Dr. Marina Shirmanova, Privolzhsky Research Medical University, Nizhny Novgorod (Russia) - Combination of metabolic imaging and genetically encoded sensors for cancer research
8. Prof. Chris Xu, Cornell University, New York (USA) - The possibility of resonance-enhanced multiphoton excitation.
9. Prof. Conor Evans, Harvard Medical School, Boston (USA) - Multiphoton Chemical Imaging to Assess Dermal Pharmacokinetics and Pharmacodynamics.
10. Prof. Karsten Koenig, Saarland University, Saarbruecken and CEO of JenLab (Germany) - Novel multimodal multiphoton tomograph based on an ultracompact femtosecond fiber laser.

Some of the most valuable contributions in this volume are articles written by highly experienced practitioners of multiphoton microscopy. They have enumerated the most important considerations in designing multiphoton microscopes and imaging experiments. Further, updates on the state-of-the-art commercial multiphoton microscope systems are presented. This volume also includes articles describing some recent advances in major multiphoton microscope components and applications including laser light sources, ultra-fast optics, filters, FRET, FLIM, FCS, Raman, CARS, SRS and Coherent Raman microscopy and spectroscopy, single molecule, endoscopy, In Vivo/Intravital imaging, metabolism measurements

including NADH, FAD, tryptophan in cells and tissues and various scientific and clinical applications.

On a personal note, the conference chairs are grateful for the participation of all authors, session chairs and acknowledge the innovation-driven manufacturers and sponsors of this conference [Applied Scientific Instruments (ASI), Becker & Hickl, Carl Zeiss, Chroma Technology, Coherent, ISS Inc., Excelitas Technologies, JenLab, LaVision BioTec, Leica Microsystems, PicoQuant, Semrock (IDEX), Spectra Physics & Newport (mks company)] and ThorLabs for their enthusiastic support in organizing this conference . We look forward to other exciting conferences in the future and welcome your continued participation and support.

Note: Late Prof. Watt Webb, Cornell University, New York, passed away in October 2020 (1927-2020). He is the inventor of multiphoton micrsocopy for biological sciences. Prof. Webb is the pioneer in methods for imaging living biological systems. He gave a Keynote lecture in the opening ceremony of the Multiphoton Microoscpy in the Biomedical Sciences in 2001 and later in 2009. We plan to have a one day speical session to celelbrate his achievements during Janaury 22-27, 2022 at the Photonics West meeting, Moscone Convention Center, San Franicsio.

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