

# ***Multiphoton Microscopy in the Biomedical Sciences XX***

**Ammasi Periasamy**

**Peter T. So**

**Karsten König**

*Editors*

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## Introduction

Thirty years have passed since the realization of two-photon laser scanning microscopy. This conference has been running for the last 20 years. Our conference at the SPIE BIOS 2020 celebrated the 20 years of this conference and 30 years achievements of the multiphoton microscopy in the biomedical sciences. The two laser companies greatly appreciated for their gift to the participants to celebrate the events. We also had a special issue on this topic in the journal of Biomedical Optics and the three conference Chairs served as Guest Editors.

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<sup>1</sup>. Two-photon excited fluorescence was finally demonstrated by Kaiser and Garrett<sup>2</sup> shortly after the invention of the laser in 1960. Sheppard and co-workers developed the SHG microscope<sup>3</sup> for solid state specimens and Denk et al. developed a non-linear fluorescence microscope and first showed that it can be applied to image biological system noninvasively<sup>4</sup>. Laser scanning multiphoton fluorescence microscopy was developed by Denk et al. in 1990<sup>4</sup>. Since then, the usage and the development of multiphoton microscopy has increased tremendously (see Fig. 1). 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<sup>5</sup>.

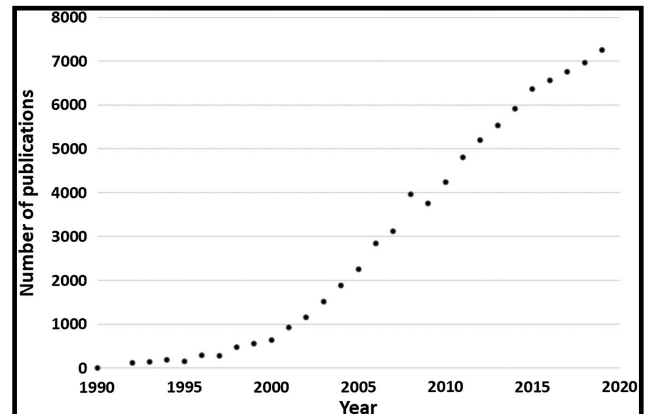


Figure 1. Number of publications in multiphoton microscopy since 1990. Adapted from JBO 2020 special issue<sup>5</sup>.

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 BIOS2020 meeting continues to address this need.

This is the 20<sup>th</sup> year of this conference and we start our conference with four Keynote lectures from leaders from various field in the multiphoton microscopy development and applications. More importantly, about 30 invited speakers from around the world shared their ideas and achievements using this novel technology.

(1) Winfried Denk, Max-Plank-Institut für Neurobiologie (Germany). **Leaving any number of photons behind: Adventures in structural neurobiology**

(2) Paras N. Prasad, University at Buffalo (United States), **Evolution of multiphoton microscopy over three decades: Current perspectives and future directions**

(3) Enrico Gratton, University of California at Irvine (United States), **Spectral phasors by the sin-cos filter method in a two-photon excitation microscope**

For the 8<sup>th</sup> year, the conference is extremely pleased to have the JenLab Young Investigator Award, in addition to our regular poster awards. JenLab Young Investigator Award is sponsored by JenLab GmbH, Germany. The award selection committee includes Drs. Arnd Krueger (Newport-Spectra Physics, USA), Paul Campagnola, University of Wisconsin-Madison, USA, Conor Evans, Massachusetts General Hospital, USA, Alberto Diaspro, Istituto Italiano di Tecnologia (Italy), Holly Aaron, University of California at Berkeley, USA and the three (Ammasi, Karsten & Peter) conference chairs. The selection process reviewing seven abstracts, manuscripts and five minutes oral presentations. The winner of the JenLab award is:

**Ms. Liubov E. Shirmolina, Privolzhsky Research Medical University (Russian Federation)**  
Imaging Plasma membrane microviscosity in cancer cells during chemotherapy.

The winner received a certificate \$2000. The award was presented by Prof. Dr. Karsten König, Saarland University, Saarbrücken, and President and Founder of JenLab GmbH, Germany.

For 20 years in a row, the conference organized poster awards for the students and postdoctoral fellows. The poster award was donated by all the conference sponsors as acknowledged at the bottom of the page. The review panels are, Holly Aaron, University of California at Berkeley (USA), Lingyan Shi, University of California at San Diego (USA), Paolo Bianchini, Istituto Italiano di Tecnologia (Italy), Alzbeta M. Chorvatova, International Laser Center (Slovakia).

The 3 poster award winners are

**(1) Reddikumar Maddipatla, Patrice Tankam, Indiana Univ. (United States)**  
Bleed-through elimination method in a dual-channel fluorescence microscopy system

**(2) Che-Wei Chang, Univ. of California, Davis (United States)**  
Monitoring drug induced changes in cardiomyocyte contractility with second harmonic generation (SHG) microscopy

**(3) Yuhao Yuan, Niraj Shah, Fa-Ke Lu, Binghamton Univ. (United States)**

Monitoring uptake of palmitic acid by glioma cells using stimulated Raman scattering microscopy

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 successfully for the last 19 years. We look forward to other exciting conferences in the future and welcome your continued participation and support.

**Editors**

Ammasi Periasamy, Ph.D.

Karsten König, Ph.D.

Peter So, Ph.D.

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