Label-Free Single-shot Multimodal Multiphoton Microscopy of Vital Samples

Haohua Tu[1,2] and Stephen Boppart[1]
[1]Beckman Institute for Advanced Science and Technology
University of Illinois at Urbana-Champaign, IL 61801 USA
[2]LiveBx LLC.
60 Hazelwood Drive, Champaign IL 61820 USA
E-mail: htu@illinois.edu

Laser-scanning nonlinear optical microscopy can acquire images at subcellular resolution and relatively large depth free of sophisticated data inversion algorithms, with endogenous contrasts often indicative of authentic biological and pathological states. Our previous works have demonstrated Simultaneous (single-shot) Label-free Autofluorescence Multiharmonic (SLAM) microscopy that enables real-time simultaneous acquisition of multiple endogenous molecular contrasts under one excitation condition to generate high-content multicolor images, which contain a rich set of orthogonally segmented cellular and extracellular components [1]. The first generation of this tool has found diverse applications in basic research of cultured cells [1] and *ex vivo* tissues [2], intravital animal microscopy [3], clinical imaging in an operative room [4], and real-time artificial intelligence-assisted cancer diagnosis [5].

However, the programmable supercontinuum laser source originally developed for SLAM is not a widely accessible technology [2, 6], whereas an alternative source based on a commercial fiber laser would compromise multimodal signal collection and increase photo-damage risk [4]. To overcome this bottleneck, we show that robust user-friendly laser sources do exist to attain fast balanced multimodal imaging with low photo-damage potential, i.e., the characteristic advantages of SLAM microscopy. The new microscope built upon this laser source enhances SLAM capabilities, allowing for portable real-world imaging outside an environmentally controlled optical laboratory. For additional improvements, straightforward strategies also exist to simplify the overall electrical-mechanical-optical schematic, increase autofluorescence detection channels, resolve lifetime information of autofluorescence signals, achieve super-resolution imaging, introduce precision microsurgery, incorporate specifically labeled imaging with no interference to endogenous signals, and co-register with histology and immunochemistry. We thus envision this enhanced SLAM to become a general noninvasive imaging tool for living organisms and biopsy specimens.

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