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Editorial

Introduction to the special issue on multiphoton imaging and quantitative characterization

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We welcome the readers to the special issue, Multiphoton Imaging and Quantitative Characterization, which aims to highlight the latest achievements in optical materials, imaging techniques, quantitative methods, and potential applications. Multiphoton imaging, including two/three photon excited fluorescence (2PEF/3PEF) and second/third harmonic generation (SHG/THG), etc., is based on the optical effect of simultaneous absorption of two or more photons from a pulsed infrared laser source. After decades of development, multiphoton imaging has emerged as a powerful modality for high-resolution, deep penetration depth, non-destructive, quantitative assessments of tissue functions in both academic research and clinical applications. Furthermore, combined with diverse subsequent quantitative characterizations, multiphoton imaging can provide new insights into assessments upon cellular metabolism and extracellular matrix organization as important indicators during disease initiation and progression. In this special issue, six research papers and one review paper are presented. We sincerely hope that these papers will bring inspiration to readers interested in this field.

We first draw attention to the work on evaluation of optical materials concerning bio-photonics. Chen *et al.* designed a setup to assess Rhod-2 Atto 680, as the fluorescence bioprobe, by simultaneous measurement of both fluorescence (radiative energy process) and photo-acoustic signal (nonradiative process) respectively with gradient tryptophan concentrations as variant environment factors.[1] Both spectrum results and intensity analysis showed that the probe's fluorescence quantum efficiency was subjected to environmental factors and the probe could be accurately quantified even with fluorescence quenching due to high concentration of probe. The authors' method helps to quantitatively trace bioprobes in complex environments.

Besides bioprobes, the excitation light is also worthy of studying. Chen *et al.* explored a longer-

wavelength excitation window for large depth imaging.[2] First, the authors identified a high transmittance window at 2200 nm on excised mouse skin sample. Then, they performed volumetric THG imaging through 250 μm depth below mouse skin surface *in vivo* excited at this window with 9 mW optical power operating at 1 MHz repetition rate. Comparative analysis implied that THG imaging excited at the 1700 nm window decayed even faster than those excited at 2200 nm as imaging depth increased with *in vivo* imaging. These results validated the 2200 nm window as a new promising excitation window potential for deep-skin multiphoton microscopy.

He *et al.* conducted three-photon excitation fluorescence (3PEF) imaging on lipid droplets (LDs) in fat liver specimens with a luminogen named NAP-CF_3.[3] A large imaging depth of 80 μm with high resolution was achieved owing to the luminogen's large three-photon absorption cross section (1.67×10^{-79} $cm^6 s^2$) and high specificity of staining on LDs. Furthermore, subsequent analysis on fat liver diagnosis at early stage was successfully carried out with excellent performance, indicating potential for future LDs-associated pathologies research.

In practice, 2PEF and SHG are often combined to attain a comprehensive understanding of the examined target. Liu *et al.* used label-free multiphoton microscopy (MPM) of both 2PEF and SHG to perform large-scale imaging on early invasive breast cancer during the transition from tumor boundary to normal tissue, and significant differences in collagen morphology were identified.[4] Further, with the assistance of automatic collagen feature extraction, eight collagen fiber morphological features were assessed to quantify the variation trend of collagen in tumor boundary, near tumor transition region and normal tissue regions. Their work may provide information for optimal resection range of breast-conserving surgery and an understanding of tumor metastasis concerning tumor-collagen interaction.

The remodeling organization of collagen fibers in extracellular matrix (ECM) can also be quantified by other approaches in other scenarios. Zhou *et al.* applied 3D alignment analysis on the spatial organization of collagen fibers within mice cervical tissues during normal gestation period with 3D SHG imaging results.[5] They found that 3D directional variance, as a novel metric of disorder in fiber alignment, peaked on day 9 in pregnancy period. The depth-dependent variation of 3D directional variance was also investigated. Subsequently, a high level of classification accuracy in distinguishing different pregnancy periods was achieved with the results of this metric. This work demonstrated that 3D directional variance was competent in monitoring the remodeling of collagen fibers within cervical tissues, hopefully allowing detection of preterm birth risk and facilitation of therapies for intervention.

Xu *et al.* presented a two-branch LGNet architecture, as a semantic segmentation network aiming to extract both local information (with convolution layers) and global features (with transformer layers) for medical image segmentation.[6] Specifically, they bridged the two types of layers and presented a novel multi-feature fusion model (MSFFM) to leverage information based on results from both branches. Finally, they validated LGNet's outperformance concerning accuracy on several medical image segmentation benchmarks and its competitive trade-off between efficiency and accuracy in comparison with other networks.

In the end, we include a review from Wang *et al.* on a wide range of methodologies that quantified organizational and morphological features of fibrous biological structures, including orientation, alignment, waviness, and thickness.[7] Each method was explained and illustrated in technical and principle details, demonstrated with specific applications in respective scenarios, and commented on the pros and cons. Perspectives of future quantification techniques were also explored.

As the guest editors of the special issue on Multiphoton Imaging and Quantitative Characterization of Journal of Innovative Optical Health Sciences, we sincerely appreciate this exceptional opportunity provided by the journal for shedding light on this comprehensive topic. Meanwhile, we would like to show our gratitude to all contributing authors for their endeavor and sharing upon their valuable knowledge and work in their subdivisions of research concerning the topic.

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