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When Maria Goeppert-Mayer made theoretical prediction of the two-photon absorption process over eighty years ago, little could be foreseen of the revolutionary breakthroughs her prediction would contribute to optical microscopy in biological sciences. However, in the past few decades, one of the most exciting developments in optical microscopy has been the extension of nonlinear optical phenomena into the realm of biological imaging.

While multiphoton fluorescence excitation has been widely applied for imaging a wide range of extrinsic and intrinsic fluorophores, harmonic generation proved that label-free imaging of tissue constituents can be achieved. Later, Raman-based technique, such as coherent anti-Stokes Raman microscopy (CARS), was uniquely used for probing vibrational properties for imaging purposes. Compared to optical microscopic imaging based on linear interaction of excitation photons with molecules, nonlinear microscopy shares a number of distinct advantages.

First, since nonlinear molecular interaction only occurs near the focal volume, images with excellent axial depth discrimination can be achieved without the use of confocal aperture. In addition, the limited excitation volume also significantly limits specimen photodamage. This central feature allowed prolonged imaging of biological specimens, rendering multiphoton microscopy the preferred technique in optical microscopic examination of tissues in three-dimensions and allowing a wide array of problems to be studied *in vivo*. Finally, a wide range of fluorescent molecules can be simultaneously excited by the use of a single excitation wavelength, allowing the simultaneous imaging of molecular species.

For example, DAPI, fluorescein, and rhodamine can be excited by the use of a single multiphoton excitation wavelength of 800 nm as the visible spectrum of blue, green, and red colors can be simultaneously visualized. Furthermore, the wide spectral separation of the near-infrared wavelengths used in multiphoton imaging and the emission wavelengths of the molecular species enables the entire emission wavelength to be easily studied. Last, the near-infrared photons commonly used in nonlinear optical microscopy are absorbed and scattered less by tissues. The enhanced image penetration depth enables in-depth imaging without invasive procedures. These unique features enable what started as a basic development of physical techniques to become a useful technique for addressing a wide range of imaging applications in biology and medicine. Recent development has even led to instrumentation capable of performing clinical diagnostics.

In this special section, an ensemble of papers describes the continual technical development, applications to biological imaging, and potential implications in medical diagnostics. Technically, Hu et al. (*J. Biomed. Opt.* 18 (3), 031102)

described the use of the use of second-order susceptibility as contrast mechanism for imaging noncentrosymmetric biological structures. By combining second harmonic generation imaging with Fourier analysis, Ghazaryan and coworkers demonstrated how fibers orientation can be quantified (*J. Biomed. Opt.* 18 (3), 031105). In addition, BaB₂O₄ was found to be useful for calibrating wavelength dependence in second harmonic generation of tissue (Shen et al. *J. Biomed. Opt.* 18 (3), 031109).

For biological imaging, two-photon lifetime imaging was applied for melanin imaging (Krasieva; et al. *J. Biomed. Opt.* 18 (3), 031107). Furthermore, Yang et al. (*J. Biomed. Opt.* 18 (3), 031104) showed that cell diagnosis in mouse cochlea can be achieved with two-photon microscopy, and infection was studied in two and three dimensions with ultrashort pulse microscopy by Gibbs et al. (*J. Biomed. Opt.* 18 (3), 031111). In medical optics, Burke et al. (*J. Biomed. Opt.* 18 (3), 031106) studied matrix alterations due to the progression of breast tumors, and Zhuang and collaborators (*J. Biomed. Opt.* 18 (3), 031103) used micro-Raman spectroscopy in studying renal tumors. Moreover, Yasui et al. (*J. Biomed. Opt.* 18 (3), 031108) used 1250 nm as the light source to induce second harmonic generation from skin dermis to study skin aging. Finally, ultrasound and nonlinear optical imaging was combined to study the structure of the human cervix (Reusch et al. (*J. Biomed. Opt.* 18 (3), 031110)). The manuscripts described in this special section demonstrate the wide array of problems researchers can address with multiphoton technology. With continual technical development, one can expect additional biological and medical applications to be found in the future.

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