Simultaneous two-photon-excited autofluorescence and second harmonic generation imaging by supercontinuum light

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Real-time monitoring the variation of chlorophyll distributions and cellular structures in leaves during plant growth provides the important information for understanding the physiological statuses of plants [1]. Two-photon-excited autofluorescence imaging and second harmonic generation imaging of leaves can be used for monitoring the nature intrinsic fluorophores distribution and cellular structures of leaves by the use of the near infra-red region of light which has minimal light absorption by endogenous molecules and thus increases tissue penetration. However the two-photon absorption peak wavelength of intrinsic fluorophores of a ficus benjamina leaf is 50 nm away from the second harmonic generation excitation wavelength, which cannot be effectively excited by a femtosecond laser beam with one central wavelength. The paper shows that a highly polarized supercontinuum light generated from a birefringent nonlinear photonic crystal fibre can effectively excite two-photon autofluorescence as well as second harmonic generation signals for simultaneously monitoring intrinsic fluorophore distributions and non-centrosymmetric structures of leaves. The 3D autofluorescence combined with second harmonic generation imaging offers scientists the useful information for studying of bio-phenomena of plants.

Figure 1  (a) An area (circled) of a ficus benjamina leaf was excited by supercontinuum. (b) Two-photon-excited autofluorescence (red) and second harmonic generation (green) image of the leaf.

References