High resolution multiphoton microscopy with low power continuous wave laser pump

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Multiphoton microscopy (MPM) is a widely used method for the imaging of scattering biological samples with large depth [1]. The key of MPM is to utilize the cascade transitions through intermediate states, where two or more photons are absorbed simultaneously. However, due to the small absorption cross-section of a multiphoton process, the MPM is usually pumped by high power laser pulse.

Here, we applied a three-photon excitation on NV center in diamond to demonstrate the high resolution MPM. Low power CW 532 and 589 nm laser were applied to pump the multiphoton charge state conversion of NV center. The wavelength of pumping laser was comparable with the fluorescence wavelength (650 - 750 nm). Unlike the traditional multiphoton process through virtual intermediate states, the charge state conversion pumped by visible light is the multiphoton transition through real intermediate states [2]. With 40 μW excitation laser, a lateral resolution of 210 nm and an axial resolution of 500 nm were achieved. It was 1.5 times of that with single photon pumped confocal microscopy. As NV center in diamond has been proved to be an excellent quantum sensor, the NV center MPM can be used for high resolution imaging and sensing in physical and biological applications with low photodamage and heating.

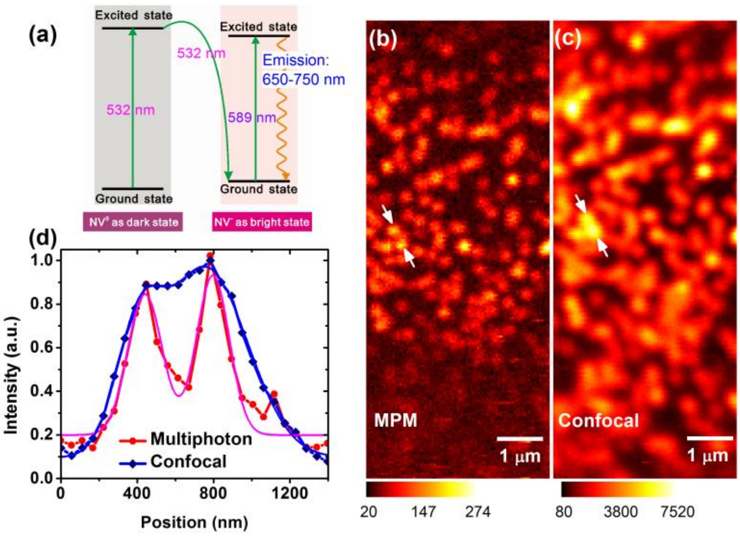

Figure 1, (a) Illustration of the multiphoton charge state transition of NV. (b) MPM and (c) confocal images of NV centers. (d) The cross-sections of the arrows in (b)(c).

Reference:

[1] F. Helmchen and W. Denk, Nat. Methods 2, 932(2005);

[2] XD Chen, S Li, et al., *Phys. Rev. Appl.* 7, 014008(2017).