

NONLINEAR ABSORPTION MICROSCOPY

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For the past two decades, nonlinear microscopy has been developed to overcome the scattering problem in thick tissue imaging. Owing to its increased imaging depth and high spatial resolution, nonlinear microscopy becomes the first choice for imaging living tissues. The use of nonlinear optical effects not only facilitates the signal originating from an extremely small volume defined by light focusing but also provides novel contrast mechanisms with molecular specificity. Nonlinear absorption is a nonlinear optical effect in which the absorption coefficient depends on excitation intensity. As a commonly used spectroscopy tool, nonlinear absorption measurement uncovers many photophysical and photochemical processes correlated with electronic states of molecules; as such the signals from various processes, such as optical bleaching, absorption saturation, stimulated emission, excited state absorption (ESA) and two-photon absorption (TPA), may provide the richness of contrast mechanisms for microscopy imaging. In this report, we summarize the recent advances on adapting this spectroscopy method to an imaging technique—nonlinear absorption microscopy, which has been demonstrated as a powerful tool to reveal the non-fluorescent chromophores encountered in imaging skin pigmentation and microvasculature[1].

The challenge of nonlinear absorption measurement is to identify a weak signal buried in a relative large background; the signal to background ratio may often be smaller than 10^{-4} . The strategy to measure such a small signal is to find ways to shift the signal out of the frequency band where the background resides. By using this “trick” we successfully extract the weak nonlinear absorption signals in either a single beam method or a double beam method with two different wavelengths (the pump-probe method). The single beam method is suitable for measuring TPA or TPA-like ESA. In the single-beam method, one pulse train from a mode-locked laser is sinusoidally amplitude-modulated at one frequency; any loss due to linear effects, such as absorption and scattering, will stay at that frequency; however the nonlinear processes, such as TPA, will distort the sinusoidal modulation; new frequency components will be generated. As a result, TPA can be measured at the new frequency component appeared at the second harmonic of the modulation frequency. The double-beam method resembles the pump-probe transient absorption measurement: If the pump pulse train is intensity-modulated at one frequency, the probe pulse train will catch that modulation if the nonlinear absorption processes depend on presence of both beams. This method can measure not only TPA and ESA but also differentiate them from the temporal behaviors when changing the interpulse delays; besides optical bleaching and stimulated emission can be also measured.

[1] Ye, T., D. Fu, W.S. Warren, (Invited Review) “Nonlinear absorption microscopy,” *Photochemistry and Photobiology*, **85**, 631-645 (2009)