Two-photon excitation autofluorescence imaging of microvasculature *in vivo*

Ting Wu, Jiuling Liao, Hui Li, Wei Zheng*

Research Laboratory for Biomedical Optics and Molecular Imaging, Shenzhen Key Laboratory for Molecular Imaging, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China;
*zhengwei@siat.ac.cn

KEY WORDS: two-photon microscopy, autofluorescence imaging, tryptophan, hemoglobin, microvasculature

ABSTRACT: Two-photon excitation fluorescence microscopy (TPM), which can provide three-dimensional subcellular imaging capability of tissues, has been proved essential for biomedical studies. TPM of endogenous fluorophores has the remarkable advantage of an unperturbed environment during investigation, especially in complex biological systems such as living cells and tissues, and thus attracted much attentions in recent decades. Previous autofluorescence TPM studies usually focused on the visible endogenous fluorophores such as NADH, FAD and so on, because these fluorophores can be efficiently excited by commercial Ti:Sapphire lasers [1, 2]. However, TPM imaging of ultraviolet endogenous fluorophores such as tryptophan, tyrosine and phenylalanine is challenged due to the lack of suitable short-wavelengths two-photon excitation light sources [3]. To address this problem, we developed a simple and robust femtosecond laser source. Its wavelength is located at 520 nm and its pulse duration is 106 fs, which are extremely suitable for TPM. With the help of this fs laser, a TPM system for autofluorescence imaging of ultraviolet endogenous fluorophores was instrumented and the autofluorescence TPM imaging of microvasculature was investigated. In this report, we will present the imaging characteristics of our TPM system and demonstrate that using this system, we could achieve intravital high-resolution 3D imaging of microvascular network noninvasively. And we could identify tumorous blood vessels from normal vessels in a mouse tumor model. This imaging capability has significant potential value for biomedical applications in future.

Reference:

[1] Xi Li, Hui Li, Xingzhen He, Tingai Chen, Xianyuan Xia, Chunxia Yang, and Wei Zheng, "Spectrum- and time-resolved endogenous multiphoton signals reveal quantitative differentiation of premalignant and malignant gastric mucosa", *Biomedical Optics Express*, V9(2), 453-471 (2018).

[2]Shenkun Zheng, Wei Zheng, Shuxia Li, Dong Li, Yan Zeng, Yanqi Yang, Jianan Y. Qu. "Multimodal nonlinear optical microscopy improves the accuracy of early diagnosis of squamous intraepithelial neoplasia", *Journal of Biomedical Optics*, V.18, 036001 (2013).

[3] Dong Li, Wei Zheng and Jianan Y. Qu, "Imaging of epithelial tissue in vivo based on excitation of multiple endogenous nonlinear optical signals", *Optics Letters*, V.34, 2853-2855 (2009).