Three-photon imaging of various mouse organ structures

Asylkhan Rakhymzhan[1], Judith Heidelin[2], Anja E. Hauser[1,3], Raluca A. Niesner[1,4]

1 German Rheumatism Research Center, Berlin, Germany
2 LaVision BioTec GmbH, a Miltenyi Biotec Company, Bielefeld, Germany
3 Immundynamics, Charité – Universitätsmedizin Berlin, Germany
4 Free University, Berlin, Germany
E-mail: asylkhan.rakhymzhan@drfz.de

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Two-photon microscopy is a breakthrough technique that got imaging of live cells to a whole new level. Based on the nonlinear nature of two-photon excitation of fluorescence markers and on the application of far-red and near infrared lasers the detection of cells deep within intact tissue in live animals has been achieved. In order to provide effective two-photon excitation, a combination of TiSa and OPO laser sources is routinely used for in vivo imaging of different cells expressing various fluorescent markers from blue to far-red and infrared fluorescence proteins. Although two-photon microscopy allows deep tissue imaging, it yields relatively poor resolution and low penetration depth when used to image through optically dense tissue, like bone, spleen or lymph node. For this reason, three-photon microscopy represents a better approach that increases the imaging depth in such tissues because of weaker scattering at higher excitation wavelengths and better contrast due to higher-order of nonlinear excitation.

Here, we demonstrate three-photon imaging of different transgenic mouse organs (brain, skull, spleen, skin, bone tissue etc.) and compare these results with the two-photon microscopy approach. We also focus on the great potential of label-free imaging techniques by detecting SHG and THG of different structures in mouse tissue. To enable three-photon excitation of green and red fluorescent proteins along with third-harmonic generation, a new optical parametric amplifier (OPA, Coherent) at 2MHz repetition rate is used. Particular interest was invested to characterize OPA laser pulses by measuring pulse widths, lateral and axial resolution. The demonstrated technique will open a new horizon in intravital deep imaging of highly scattering tissues.