IN VIVO ANALYSIS OF LYMPHOCYTE TRAFFICKING USING MULTIPHOTON MICROSCOPY AND OPTICAL PROJECTION TOMOGRAPHY

Jens V. Stein, Ph. D., Varsha Kumar, Ph. D., Silvia F. Soriano, Ph. D.

Theodor Kocher Institute

University of Bern – P24 Freiestr. 1

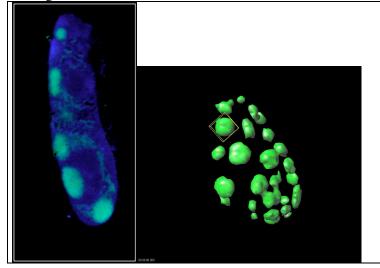
3012 Bern/Switzerland

jens.stein@tki.unibe.ch

The Theodor Kocher Institute (TKI) of the University of Bern has a longstanding history in research in inflammation, in particular concerning leukocyte migration. In concrete, our research activities center around the analysis of lymphocyte recirculation pathways and novel imaging methods. To this end, we have installed a multiphoton microscope setup at the Theodor Kocher Institute/University of Bern. This setup is equipped with a two different image acquition modes: slow, deep tissue PMT imaging using single beam excitation, and fast, low penetration multibeam excitation. Both modes are useful for different objectives in the IP, i.e. popliteal lymph node imaging and improved fast-speed intravital microscopy. From preliminary experiments, we expect deeper insights into dynamic cell movement inside lymphoid organs important for immune responses.

In collaboration with P17-1, we have established whole-mount staining techniques to identify B cell follicles in secondary lymphoid tissue (lymph nodes, spleen). These samples were analyzed using Optical Projection Tomography (OPT), which allows a three-dimensional reconstruction of labeled structures. This will allow a novel, quantitative approach to organ structure-function studies.

Figure 1:



Left panel. Virtual OPT section of a mouse lymph node after whole-mount labeling with B220-Alexa488 (green), which labels B cell follicles. Right panel. Computer-assisted 3D reconstruction of B cell follicles.