

## Workshop

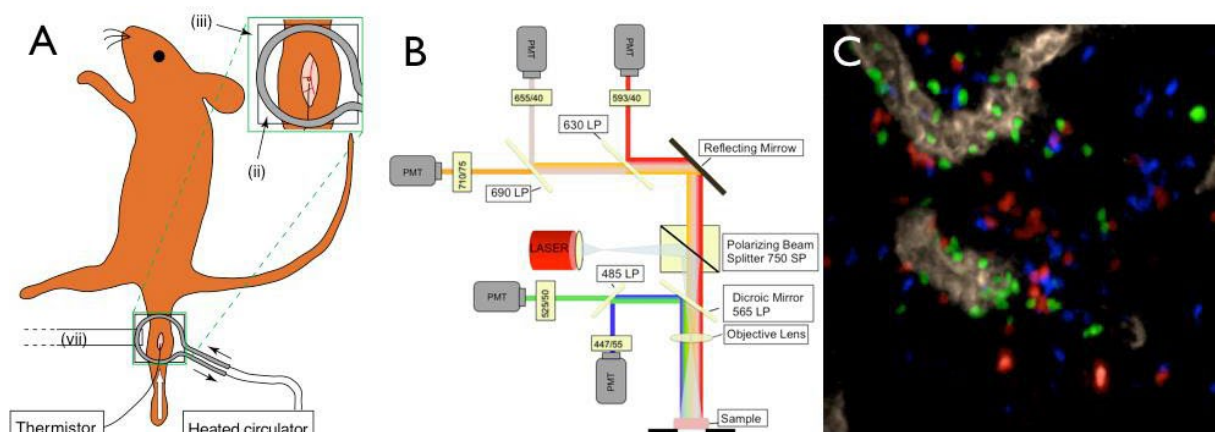
<b>Title:</b>	<b>Multiphoton Intravital Microscopy</b>
Date, duration:	October 4 <sup>th</sup> or 6 <sup>th</sup> , 2023
Location:	Chemin du Musée 5, Per-09, 1700 Fribourg
Lecturer(s):	Prof. Dr. Jens Stein (OMI)
Number of participants:	1 – 3 students
Target audience	Master (and PhD) students of the University of Bern. Students need to have passed the Lecture Series on Advanced Microscopy exam to participate (KSL 9256).
Registration:	Send request to Jens Stein ( <a href="mailto:jens.stein@unifr.ch">jens.stein@unifr.ch</a> )
KSL:	470964
Reward:	0.5 ECTS
Costs:	260 CHF (total costs per course) - PhD students enrolled in the Graduate School for Cellular and Biomedical Sciences ( <a href="#">GCB</a> ) can apply for refund at the PhD program Cutting Edge Microscopy - Amount accounts for students of the University of Bern. Other participants, please request quote.
Learning goals:	During the one-day course, students will first receive an in-depth refreshing on the basic principles of multiphoton excitation of fluorophores. Subsequently, students will be introduced into the multiphoton setup at the TKI and briefly assist the surgical preparation required for in vivo imaging. Finally, image acquisition and analysis procedures will be introduced. The overall aim is to obtain a general overview over the theoretical basics and practical procedures of MPM, and its applicability for immunological research.



Description:

Multiphoton microscopy (MPM; also sometimes called non-linear or twophoton microscopy) is the appropriate technique to observe fluorescently labeled cells deep (>50 μm) within biological tissue. In MPM, fluorophores are excited through simultaneous absorption of two (or more) low energy photons, instead of one high energy photon. Simultaneous absorption requires high photon densities, which are only present at the focal point of the laser beam. When relapsing to their ground state, excited fluorophores release photons as in common high-energy excitation. Fluorescent signals are collected for softwarebased image analysis.

The use of low-energy photons and the restricted occurrence of light absorption at the focal point confer several advantages over commonly used high energy photons. The latter are readily absorbed by tissue, resulting in low penetration depth. In addition, high-energy photons cause more phototoxic side effects and produce unwanted out-of-focus excitation. In contrast, low-energy photons penetrate deeper into tissue and excite fluorophores only in the focal plane. This allows collecting all fluorescent light and increases sensitivity (Figure 1).



**Figure 1. A.** Schematic outline of MPM of mouse popliteal lymph node (LN) model. The right leg of the mouse is fixed and the popliteal LN is surgically exposed underneath a cover slip (iii) and kept at 37°C with a heating ring (ii) attached to a fixed holder (vii) to reduce tissue movement. **B.** Customproduced MPM detection setup established at our institute for simultaneous detection of 5 epifluorescent signals. **C.** Representative 4-channel image from our MPM setup showing the interactions between control transgenic CD4<sup>+</sup> T cells (red) with dendritic cells (DC) presenting cognate peptide complexes (blue) inside a popliteal LN. Transgenic T cells lacking the promigratory factor DOCK2 (green) show increased accumulation around blood vessels (brown), resulting in reduced frequency of T cell – DC interactions.

Course structure: 1 day practical work (10 am – 5 pm)

Assessment: Q & A session at the end of the workshop