# LONGITUDINAL INTRAVITAL IMAGING OF THE BONE MARROW

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**ABSTRACT:** The bone marrow (**BM**) is a central organ of the immune system, which hosts complex interactions of bone and immune compartments, relevant for hematopoiesis, immunological memory, and bone regeneration. Although these processes take place over months or even years, existing imaging tools allow us to follow short snapshots of only a few hours. We developed a microendoscopic multi-photon imaging approach called LIMB (**L**ongitudinal intravital **IM**aging of the **B**one-marrow) to analyze cellular dynamics within this organ after bone injury and during homeostasis. The approach combines a biocompatible plate surgically fixated to the mouse femur and a gradient refractive index (**GRIN**) lens as microendoscope, allowing imaging deep within the tissue.

**LIMB:** A modified medical grade titanium implant [1] is fixed with two screws onto the mouse femoral bone (Fig. 1 a). The Endoscope tubing has an inner diameter of 450 µm and an outer diameter of 650 µm and leads into the marrow cavity. The sapphire window seals the tubing at the intramedullary end. The GRIN lens is fit into the endoscope tubing and its outer tip can be accessed by the microscope objective. A reference plate (Fig. 1 c, inset) is used for the alignment of the GRIN lens to the microscope objective. It holds the implant and the femur in a stable position under the customized imaging stage (Fig. 1 b).

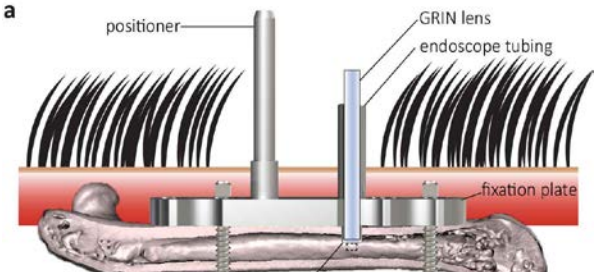
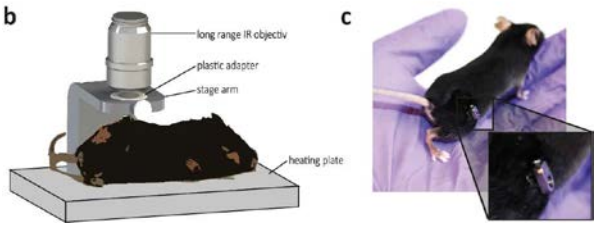


Figure 1: Assembly and application of LIMB




**RESULTS:** We scored all animals based on behavior, appearance and activity. All mice recovered within 7-10 days post surgery. Via histology, we demonstrated that the tissue in front of the imaging window is comparable to other regions after 28 days. In distinct longitudinal imaging experiments we **1.** acquired cellular motility of B lymphocytes and vessel patterns for up to 90 days, and **2.** demonstrated the positional stability by repeated photo-activation of paGFP-labled BM cells. LIMB revealed extensive vascular plasticity within the BM during bone healing and homeostasis. To our knowledge, this vascular plasticity is unique among mammalian tissues, and we expect this insight will decisively change our understanding of various processes taking place in the BM.

**REFERENCES:**

[1] R. Matthys and S.M. Perren,"Internal fixator for use in the mouse", *Injury*, 103-109 (2009).