

SINGLE CELL MANIPULATION AND IMAGING *IN VIVO* REVEALS MICRO-REGENERATION DYNAMICS OF TISSUE

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The study of regenerative processes *in vivo* requires following cells on a single cell level. However, this is difficult as histological sections are an endpoint-based analysis. Ritsma et al. have developed an abdominal imaging window, which can be used for longitudinal imaging in mice over a period of several weeks [1]. We evaluated if this method can be combined with multiphoton microscopy, which allows besides of high resolution imaging the femtosecond laser based ablation to induce micro-regenerative processes in the liver. Compared to macro-regeneration, for example, after partial hepatectomy, this approach can shed novel light on single cell dynamics.

We injected FITC dextran for labelling of vessels and Rhodamine 6G for hepatocytes in Balb/C mice with an abdominal imaging window. A combined multiphoton microscopy and amplifier-based femtosecond laser system for cell ablation was applied to induce single cell ablation during simultaneous visualization of targeted cells using multiphoton microscopy. Pulse energies in the range of 3-6.6 μJ proved to be feasible for imaging and re-localization of ablated regions in follow-up measurements after 2-7 days was possible to analyze regeneration of tissue in the same animals. The use of the amplifier system avoided heat accumulation in deeper tissue regions.

Currently, we aim to extend this micro-regeneration imaging to other organ systems, in particular, the lung. As the use of an imaging window is difficult in the lung, we are developing a fiber-based imaging method, based on a fiber-imaging bundle, and appropriate surgical approaches to be able to follow the regeneration of the same region of interest in successive imaging sessions.

- [1] L. Ritsma, E. J. a Steller, S. I. J. Ellenbroek, O. Kranenburg, I. H. M. Borel Rinkes, and J. van Rheenen, "Surgical implantation of an abdominal imaging window for intravital microscopy.," *Nat. Protoc.*, vol. 8, no. 3, pp. 583–94 (2013).