

# INTRAVITAL MULTIPHOTON FRET IMAGING OF CALPAIN PROTEASE ACTIVITY IN MICE

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## Abstract

The investigation of molecular processes *in vivo* requires dedicated imaging methods allowing visualization of protein-protein interactions, enzymatic activities and intracellular Ca<sup>++</sup> fluxes. Fluorescence resonance energy transfer (FRET) detection methods are ideally suited, but have not been adapted so far to 3D *intravital* imaging. We have combined here multiphoton microscopy (MPM), which is method of choice for non-destructive living tissue inspection, and FRET imaging to monitor calpain proteolytic activity in living mouse muscle. We engineered a FRET-based indicator of calpain activity containing eCFP and eYFP fused to the ubiquitous calpains cleavage site of  $\alpha$ -fodrin (Calpain-sensor) and expressed this transgene in mouse muscle 7 days prior to MPM. To extract accurate FRET efficiency images and convert data into knowledge, we performed corrections for depth attenuation and used acceptor photobleaching experiments to calibrate the final results. Our experiments highlights: (i) the feasibility and accuracy of three dimensional multiphoton quantitative FRET imaging *in vivo* (ii) the ability of this method to detect FRET at cellular and sub-cellular levels up to 200  $\mu$ m in living organs. This report demonstrates that numerous applications based on FRET biosensors developed in cell cultures can be applied in living animals.

## References

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