Title

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In Vivo Metabolic Mapping of Stem Cells and Differentiated Progeny in Small Intestine and Colon Crypts by Phasor Fluorescence Lifetime Microscopy

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We have performed label-free Phasor Fluorescence lifetime microscopy (FLIM) to reconstruct the three dimensional metabolic signature of small intestine and colon tissue *in vivo*. Our method provides a label-free identification and metabolic mapping of stem cells during differentiation. Freshly excised tissues are imaged with two photon microscopy and FLIM within two hours. Lgr5-GFP mice are used to mark the Lgr5+ stem cell population at the base of small intestine (SI) and colon crypts. Using Phasor FLIM analysis of live tissue excited at 880nm and 740nm, we identify and map the concentration of different intrinsic metabolic fluorophores and extracellular matrix elements such as NADH, FAD, and collagen. We observe that different compartments of the tissue are defined by unique Phasor FLIM signatures. We can distinguish collagen fibers at the base of the crypts, the lamina propria, the vascular network and the epithelium. The FLIM signature at the base of the crypt at 740nm follows exactly the map of stem cells intercalated between adjacent Paneth cells. Paneth cells are characterized by a different FLIM signature with respect to the stem cells thus indicating a difference in the concentration and/or composition of intrinsic fluorophores. The FLIM Z-stack reveals a shift of the metabolic signature of crypt epithelial cells during differentiation. Stem cells at the base of the crypt have the shortest lifetime and the highest NADH/ NAD+ ratio. Movement up the crypt to transit amplifying cells and fully differentiated cells on the mucosal surface corresponds to different FLIM signatures that correspond to decreasing NADH/ NAD+ ratios, as is expected during differentiation.

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