Three dimensional live cell imaging of intestinal organoid structures by spinning disc microscopy

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Cultivation of stem cells and three dimensional (3D) organoids represent a brilliant tool to study complex cell biological processes in vitro. Application of these methods on one hand reduces the need for animal studies and on the other hand enables scientists to investigate cellular mechanisms in more detail and over time. Beside establishing the cultivation of organoids, this technique increased the demand for the adaption of hitherto used methods for the analysis of 3D structures. Especially the increasing sizes of multi cellular formations illustrated the limitations of conventional confocal laser scanning microscopy. Both the short working distance of high magnification lenses and the low scan speed of conventional laser scanners are incompatible with successful confocal live cell imaging of huge 3D cell structures. Therefore, we adapted confocal spinning disc microscopy for live cell imaging of intestinal mouse organoids. We applied this method to image the process of cell differentiation within intestinal epithelial cells for the first time in three dimensions. Small intestinal epithelial organoids were generated from transgenic mice, expressing the green fluorescent protein (GFP) under control of the promotor for the intestinal stem cell marker Lgr5. Within these organoids, GFP and the fate of stem cells could be followed by live cell microscopy. To visualize cell division within the stem cell compartment we stained the DNA with the fluorogenic live cell dye silicon rhodamine (SiR) fused to Hoechst. Using this procedure, we were able to image the division of mouse stem cells within the intestinal epithelial layer not only at confocal resolution and in three dimensions, but also continuously for up to four days. Using this long-term 3D approach, we received a unique insight into cellular processes of the intestinal stem cell niche. Finally, a sophisticated reconstruction of confocal image stacks allowed quantification of cellular effects in three dimensions.