

# Dual illumination inverted light sheet microscope for long term live imaging

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Organoid cultures have been recently established to study organ formation and tissue morphogenesis. However, imaging of these samples has been hampered by their long and often inefficient development and their light sensitivity. Light sheet microscopy would be the imaging technique of choice due to its low phototoxicity and high acquisition speed. However, many current light sheet microscopes suffer from complicated sample mounting that also limits sample survival (e.g. mounting in FEP tube or agarose embedding) and lack of multi-position imaging. To overcome these limitations we have built an inverted light sheet microscope system with two illumination objectives, one high NA imaging objective and a beam scanning and alignment module. The sample is easily accessible and completely isolated from immersion medium and multiple samples can be imaged in parallel. Using this microscope we were able to acquire a continuous five-day long time-lapse capturing the formation of fully grown intestinal organoid starting from a single stem cell embedded in a matrigel. By imaging the stem cell marker (LGR5-GFP) we could follow for the first time the dynamics of stem cells during a complete intestinal organoid development at single cell resolution. Moreover, we demonstrated the versatility of this microscope by imaging different organoid models and the development of several living samples across different scales (e.g. zebrafish, mouse embryo and *C. elegans*).

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## Terms and Conditions

Yes

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