Automated screening by 3D light-sheet microscopy reveals mitotic phenotypes

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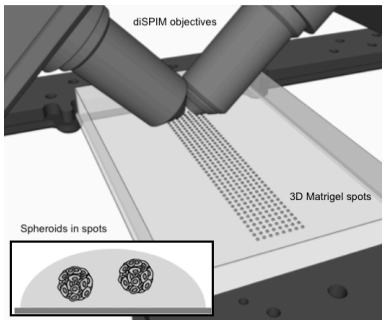
3D cell cultures enable the *in vitro* study of dynamic biological processes such as the cell cycle, but their use in high-throughput screens remains impractical with conventional fluorescent microscopy. Here, we present a screening workflow for the automated evaluation of mitotic phenotypes in 3D cell cultures by light-sheet microscopy. After sample preparation by a liquid handling robot, three-dimensional cell spheroids are imaged for 24 hours with a dual inverted selective plane illumination (diSPIM) microscope [1] with an improved signal-to-noise ratio, higher imaging speed, isotropic resolution and reduced light exposure compared to a spinning disc confocal microscope. A dedicated high-content image processing pipeline implements convolutional neural network (CNN) based phenotype classification. We illustrate the potential of our approach by siRNA knock-down and epigenetic modification of mitotic target genes for assessing their phenotypic role in mitosis. By rendering light-sheet microscopy operational for high-throughput screening applications, this workflow enables target gene characterization or drug candidate evaluation in tissue-like 3D cell culture models.



We used up to 320 spotted 3D Matrigel drops which contain differently treated grown spheroids (see schematic inlay left lower corner). diSPIM dipping objectives from top dual geometry scan for selected spheroids in time lapse. Automated CNN classification allowed detailed mitotic phenotypic analysis of various genetic and epigenetic perturbations in 3D, over time and in comparison.

Reference

[1] Y. Wu, P. Wawrzusin, J. Senseney, R. S. Fischer, R. Christensen, A. Santella, A. G. York, P. W. Winter, C. M. Waterman, Z. Bao, D. A. Colón-Ramos, M. McAuliffe, H. Shroff, "Spatially isotropic four-dimensional imaging with dual-view plane illumination microscopy" *Nature*, 31, 1032–1038 (2013).