BRING OBLIQUE ILLUMINATION DEEPER INTO SAMPLES TO ENABLE IMAGING OF LARGE 3D CELL CULTURES ON CONVENTIONAL MICROSCOPES

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Oblique Plane Microscopy, Lightsheet, Organoids, Deep Imaging

The advent of 3D cell cultures in Biomedicine, e.g. in the form of spheroids or organoids, poses a challenge to 3D imaging, in that the ideal microscope should now be able to image large multicellular tissues in fluorescence, with high-throughput, in live imaging and over long period of time (several days), and deep into the sample. Many models of organoids grow in scaffolds, which inevitably increases the need for long working distances, in direct conflict with optical resolution due to the lower numerical apertures usually available.

Lightsheet Fluorescence microscopy is now recognized for its ability to preserve samples viability for long term imaging, however the mounting strategy for 3D cell cultures is still an obstacle to high-throughput imaging. Recent efforts have shown that Oblique Plane Microscopy [1] can make use of a single objective lens to illuminate samples with a highly inclined plane of light, while detecting the image plane through 3 concatenated detection units to retrieve an in-focus image. This has shown to work on live spheroids in commercial multiwell plates on a conventional inverted microscope [2], thereby potentially and considerably simplifying high-throughput protocols. The main limit of this implementation, however, seems to reside in the working distance, somehow limiting the access to deeper volumes and impairing access to scaffold-based cell culture systems.

We have taken the challenge to extend the working distance capability of OPM and will show preliminary results showing that it can be performed down to several millimetres with reasonable 3D capability. Importantly, we combined OPM and SPIM on the same instrument to benchmark sample accessibility, resolution and depth of imaging with long working distance in tumour-derived spheroids. We will discuss strategies of illumination, multiview imaging and image restoration with this long working distance OPM set up.

[1] C. Dunsby, "Optically sectioned imaging by oblique plane microscopy". *Opt. Express* **16** (25): 20306-16 (2008)

[2] V. Maioli, G. Chennell, H. Sparks, T. Lana, S. Kumar, D. Carling, A. Sardini, C. Dunsby, "Time-Lapse 3-D measurements of a glucose biosensor in multicellular spheroids by light sheet fluorescence microscope in commercial 96-well plates" *Sci. Rep.* **6**, 37777 (2016).