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COMPARISON OF IMAGE DECONVOLUTION METHODS TO IMPROVE IMAGE QUALITY OF 3D CELL CULTURES ACQUIRED USING HCS/HTS EQUIPMENT

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Introduction and objective: Accurate imaging analysis of 3D in vitro cell cultures became critical to evaluation of morphological and some physiological aspects of cells in these aggregates. Fluorescence microscopy protocols allow analysis of a plethora of molecular markers, DNA content and integrity and assessment of morphological changes upon different conditions. Light diffraction by plastic (or glass) substrate, and through sample and mounting/culture media distorts events, and thus images must require deconvolution methods to proper visualization and analysis. The work used 2 different algorithms and 3 iteration numbers to deconvolve images of spheroids acquired in large scale using HTS/HCS equipment.
Methodology: Murine fibroblasts (NIH/3T3) were seeded on non-adhesive 96-well plates in RPMI 1640 medium. After 72 hours in culture, formed spheroids received pan-nuclear (Hoescht 33342) and nucleus of dead cells (SYTOX® Green) staining and imaged using HCS/HTS equipment (INCell 2500 HS, Cytiva). PSF files were generated using fluorescent beads in same cultured medium. Using DeconvolutionLab2 [1], a Fiji [2] plugin, spheroid images were deconvolved using the Tikhonov-Miller or Richardson-Lucy algorithms, with 10, 20 or 30 iterations. Orthogonal views of deconvolved stacks were analysed to find best results.
Results and discussion: Both algorithms rendered similar results, with good reproducibility and resolution of light distortions. No real advantages were perceived in 30-iterations experiments, although 20 iterations rendered best images, regarding shape of nuclei (must be as spherical as possible) and drastic reduction of light refraction between slices.
Conclusions: The work found that both methods were very easy to apply to stacks, with similar results. 20 iterations must be the better option to deconvolve spheroid images, uniting efficiency and saving computational resources. Richardson-Lucy can be the method of choice, as produced slightly better results.

References

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