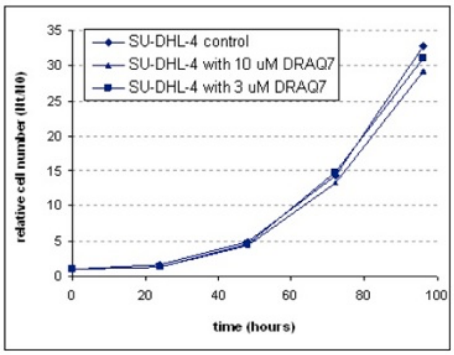
BIOSTATUS

DRAQ7™

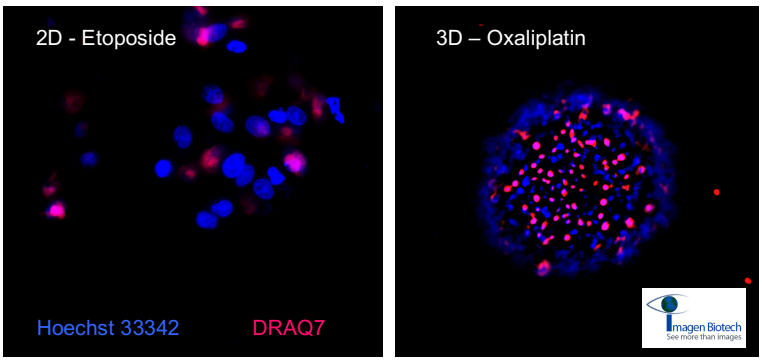
Far-Red Fluorescent Live-Cell Impermeant DNA Dye

Real-Time Cell Health Monitoring, Cell-by-Cell, in 2D and 3D in the Far-Red

In **flow cytometry** and **fluorescence microscopy** (including **high content screening**), a reliable estimation of cell viability is important because it is central to assays for **tumour cytotoxicity**, **apoptosis** and ***in vitro* toxicology**. Likewise, it is a measure of sample quality and reliable phenotypic analysis. To improve reporting of such cell viability DRAQ7™ was developed. Essentially, this water-soluble probe has identical spectral properties to DRAQ5™ avoiding overlap with vis. range fluors while RNA binding is negligible. However, DRAQ7™ is membrane impermeant, yet rapidly enters "leaky" cells to label nuclear DNA. Therefore, DRAQ7™ can be used as a new far-red reporter of cell viability and plasma membrane-permeabilization resulting from damage, apoptosis and necrosis. DRAQ7™ is an ideal spectrally-shifted replacement for DAPI, propidium iodide and TOTO-3 and may offer some new information and a wider assay window. In drug discovery and *in vitro* toxicology DRAQ7™ can be combined with live cell-permeant DNA dyes (e.g. CyTRAK Orange™ and Hoechst 33342) for live/dead counting. As an example in a typical HCS cell health assay, which combines a dye for "all events" (Hoechst 33342), a mitochondrial membrane potential reporter (TMRM) and DRAQ7™ which gives less spectral overlap and wider detection windows for mitochondrial and viability components whilst reducing assay costs compared to TOTO-3.



As shown in the graph above, cells cultured in the presence of DRAQ7™ at standard concentration or 3.3X excess show insignificant impact on growth curves compared to controls making DRAQ7™ an ideal reporter for cell death in real-time, long-term viability / toxicity assays. DRAQ7™ has been validated in key cytometry publications (Akagi, 2013a & 2013b; Wlodkowic, 2013; Smith, 2013) for multi-colour experiments and uniquely for long-term, real-time analysis. It has been used in an image-based screen to monitor cell viability in 2D and 3D spheroid / micro-tissue assays including a study on glioblastoma-derived stem cell lines in response to a library of therapeutic agents (images above; detailed in a separate BioStatus / Imagen-Biotech white paper).



Subsequently, DRAQ7™ has been applied to a variety of **long-term and real-time cell health assays** that benefit from its fundamental features: cell health in pancreas tissue-culture (7 day assay; Marciniak, 2013), nano-particle toxicity (40+ h; Ware, 2014), mitochondrially-regulated apoptosis (72 h; Liang, 2015), cell number expansion versus cell death in tumor inhibition (72 h; Stratikopoulos, 2015), mitochondrial-size linkage to BAX-dependent apoptosis (18 h; Renault, 2015) and host-directed therapy to eliminate *Legionella* (72 h; Speir, 2016).

Significantly, in cancer therapy research, use of DRAQ7™ has shown the importance of discriminating between cytostatic and cytotoxic anti-tumour effects since experiments show these can be independent of 3-D spheroid diameter (36h; Dunlop, 2017), where after removal of cytostatic treatment spheroid growth may recover.

Previous methodologies have positively marked cells based on metabolic competence. Conversely, DRAQ7™ labels only membrane-compromised (damaged, dying, dead) cells, red excitation minimises risk of DNA damage when capturing multiple time-lapse images whilst reliably monitoring viability in real-time, cell-by-cell.

DRAQ7™ offers new dimensions and opportunities for performance of high value phenotypic and *in vitro* toxicity cell-based assays in drug discovery and development, that can be applied across different platforms including flow cytometry, imaging flow cytometry, fluorescence microscopy and high content imaging platforms.

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