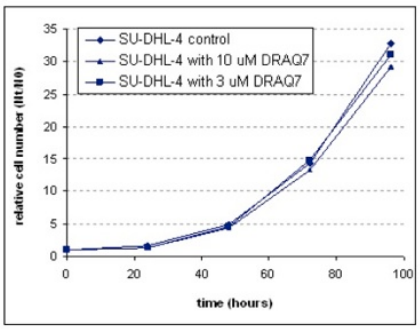
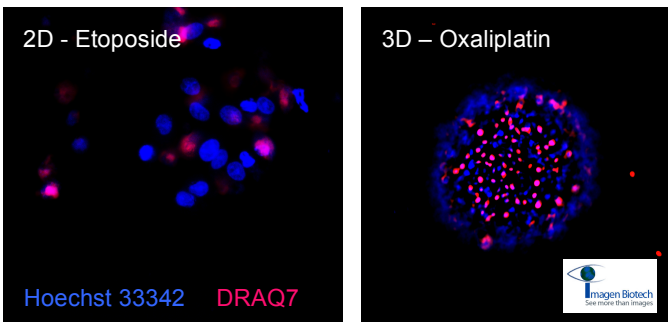
Real-Time Cell Health Monitoring, Cell-by-Cell, in 2D and 3D in the Far-Red.

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 **apoptosis** and ***in vitro* toxicology**. Likewise, it is often a useful measure for sample quality and for robust phenotypic analysis of clinical samples. To better report such cell viability DRAQ7™ (Edward, 2011) was developed, based on DRAQ5™. Essentially, this water-soluble probe has identical spectral properties to DRAQ5™ and as such does not overlap with the majority of visible range fluors. Likewise, RNA binding is very weak (undetectable by flow cytometry). However, the chemical modification has rendered DRAQ7™ membrane impermeant, thus it does not cross the membrane of viable cells but rapidly enters "leaky" cells and labels nuclear DNA. Therefore, DRAQ7™ can be used as a new far-red reporter of cell viability and conversely cell membrane-permeabilization resulting from damage, apoptosis and necrosis. As such, DRAQ7™ is an ideal spectrally-shifted replacement for agents such as DAPI, propidium iodide and TOTO-3 and may offer some new information and a wider assay window. For HCS studies in drug discovery and *in vitro* toxicology DRAQ7™ can be applied as a reporter of cell-membrane permeabilization, combined with live cell-permeant DNA dyes (e.g. CyTRAK Orange™ and Hoechst dyes). DRAQ7™ can be applied as a viability reporter in mitotic index assays and in studies into cell health in response to insults. As an example, for the typical HCS cell health assay, which combines a dye for "all events" (Hoechst 33342), a mitochondrial membrane potential reporter (e.g. TMRM) and a cell viability dye (TOTO-3), the latter component can be replaced with DRAQ7™ for less spectral overlap and thus wider detection windows for the TMRM and cell viability components whilst significantly reducing total assay reagent costs.



As shown left, SU-DHL-4 cells cultured in the presence of DRAQ7™ at both the standard concentration or 3.3X excess show no significant impact on growth curves compared to untreated controls making DRAQ7™ an ideal candidate as a reporter of cell death in real-time, long-term viability and toxicity assays. DRAQ7™ has been robustly tested and exemplified in key cytometry publications (Akagi et al, 2013a; Akagi et al, 2013b; Wlodkowic et al, 2013; Smith et al, 2013) for compatibility with multi-colour experiments and uniquely for long-term, real-time analysis. It has recently been utilised in imaging procedures to monitor cell viability in 2-D and 3-D spheroid/micro-tissue assays including a study on glioblastoma-derived stem cell lines in response to a library of chemotherapeutic agents (shown below and detailed elsewhere in a separate joint BioStatus / Imagen-Biotech white paper).



Additionally, DRAQ7™ has been applied to a variety of **long-term and real-time cell health assays** that benefit from its fundamental features. For example, DRAQ7™ has been applied to monitoring cell health in 7-day culture of pancreas tissue (Marciniak et al, 2013), for real-time nanoparticle toxicity monitoring (Ware et al, 2014), for real-time study of mitochondrially-regulated apoptosis (Liang et al, 2015) and detecting sub-cutaneous extracellular dsDNA in better understanding its adjuvant role in vaccination (Wang et al, 2015).

Previous methodologies have attempted to positively mark cells based on metabolic competence. DRAQ7™ labels only (damaged, dying and dead) membrane-compromised cells and, importantly, red excitation minimises risk of DNA damage when capturing 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, fluorescence microscopy and high content imaging platforms.

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If you would like to know more about DRAQ7™ or any other BioStatus product get in touch ..

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