Using DRAQ5™ in HCS – what can you see?

DRAQ5™ has been used widely in high content screening and related drug discovery assays for more than a decade. Recent papers give a clear overview of the different intracellular features one can render with DRAQ5™ staining.

Let's start with the basics; far-red fluorescing DRAQ5™ can be used on live- or fixed-cell endpoint assays giving excellent flexibility in assay development. High photo- and chemical-stability allow it to be prepared in large quantities for unattended operation in automated workflows while it can be admixed with formaldehyde for a single-addition fix-&-stain step, for example in GFP translocation assays and intracellular pathogen detection.

At its simplest, the stoichiometric labelling of dsDNA by DRAQ5™ has made it the DNA dye of choice for whole well or "in-cell western" assays performed on the LiCor Odyssey (e.g. Ilg, 2018) and TTP Labtech Mirrorball (e.g. Rossotti, 2018) platforms in hundreds of published papers. DRAQ5™ reports the number of cells in such whole-well assays and thereby corrects the protein expression level for well-to-well cell number variances across a microtiterplate. This has been validated to perform with good linearity at 5 µM, creating a standard curve for fluorescence versus cell number per well.

Object identification by nuclear counterstaining

In image-based high content assays individual cells are typically identified using a nuclear counterstain. DRAQ5™ has been widely described for this initial "object identification" process in hundreds of papers, due to its reliability, spectral characteristics and core features described above. Thereafter, one can clearly segment the nucleus based on DRAQ5™ staining, to allow creation of a mask from which it is possible to dilate a region around that mask as "cytoplasm" for the determination of protein expression change, a translocation event (Brightbill, 2018), for example, pro-apoptotic response in cancer therapy (Milazzo, 2017) or a cell texture or organellar alteration (Thongon, 2018) due to compound treatment, gain-of-function (Kim, 2017), RNAi knockdown (Polajnar, 2017), etc. that might be sentinel in a phenotypic approach. Not least, the fluorescence information about the nucleus from DRAQ5™ can be combined with brightfield or phase contrast information.

Cell cycle position / DNA content information

The fluorescence intensity of each masked nucleus can be used to give population-level information about the impact of a treatment on the proliferation of the cells in that well relative to vehicle and controls, and displayed as histograms analogous to flow cytometry. The condensation of individual nuclei or nuclear fragmentation can also be used to infer apoptosis (Bourton 2017).

Cell-Cell Adherence Assays

Marking populations of cells for a functional adhesion assay makes deconvolution of the assay much simpler. In one example of this, live macrophage-like MM6 cells were labelled with DRAQ5™ and allowed to adhere to endothelial cells (Hoechst 33342-labelled) which had been exposed to a cigarette smoke extract or controls to assess the impact of the cigarette smoke extract on vascular inflammation and atherogenesis (Poussin, 2015).

Single-channel segmentation of nucleus and cytoplasm

Importantly, what has made DRAQ5™ the go-to nuclear counterstain for high content assays in drug discovery is the secondary staining that can be detected in the cytoplasm.

One segments on the nucleus, creates a mask and then uses a "watershed" or similar algorithm to delineate the cytoplasmic region. This is much more representative of the true boundary of the cell compared to the pixel-dilation for an assumed "region of interest" (as described above) and maybe more reliable when considering cells with variable morphology, perhaps as a consequence of cytotoxic effects. This powerfully improves the useful information available from each cell object for a translocation, protein expression change or other intracellular event (Schafer, 2018; Demers, 2018). Usefully, this two-compartment segmentation with DRAQ5™ comes from only one fluorescence channel giving further freedom in assay design.

The above features can be similarly applied to assays in imaging flow cytometry, for example, measurement of volumetric changes to cells, e.g. combining DRAQ5™ and a separate cytoplasmic stain such as calcein (Adlung, 2017).

Detecting DNA-bearing intracellular parasites and other inclusions

In assays for the search for treatments against intracellular parasites such as trypanosomes and leishmania one can use cell number (nuclear count) to measure host-cell toxicity, two-compartment segmentation to identify individual cell objects and then delineate the cytoplasm. Within the cytoplasm one can detect and enumerate the parasite infection of each cell (Demers, 2018; Yang, 2017) or phagocytosed bacteria as bright punctate spots of DRAQ5™ staining.

Measuring unwanted toxicity

The biggest risk to drug development remains unwanted toxicity and related side-effects. DRAQ5™-based assays can provide a number of useful readouts which indicate cytotoxicity. A reduction in cell numbers (i.e. nuclear count) is the most obvious and immediate feature one can correlate to unwanted toxicity (Cogo, 2018) and, independently, the change in structure and fluorescence characteristics of the nuclei (Simonen, 2008; Bourton, 2018) which includes condensation and fragmentation, often associated with apoptosis.

An extension of this can be the combination of Calcein Green AM staining of (live) cells with DRAQ5™ (all cells) to determine a live:dead ratio. In one example, this was done on colonospheres to measure the effectiveness of an anti-cancer treatment (Trumpi, 2015).

Additionally, the dual compartment staining of the nucleus and cytoplasm by DRAQ5™ contains an enormous amount of additional information that can infer unwanted or idiosyncratic cytotoxicity. Multiple measurements of the cytoplasmic and nuclear regions can then allow an unbiased interrogation of these changes and their association with cytotoxicity and the usefulness of this has been well described in the search for new anti-virals (Berke, 2010) and anti-sense oligonucleotide drugs (Sewing, 2018) where object shape change was compared against vehicle and controls.

Uncovering genotoxicity

A requirement for all new therapeutics is demonstrating a lack of genotoxicity. A central assay for this is micronucleus detection test. This is typically done late in the discovery phase due to the cost and complexity of the assay. However, developments show that it is possible to automate this test to bring it further up the "pipeline". In the first instance, Hoechst 33342 and DRAQ5™ were combined to enable an automated microscopy-based methodology (Westerink, 2011).

More recently, DRAQ5™ has been demonstrated on the imaging flow cytometry platforms to allow reporting and enumeration per cell of micronucleus events following a simulated radiological event exposure of whole blood using the Merck Imagestream® (Rodrigues, 2014). Following this work, a practical and cost-effective automation of large-scale micronucleus detection for drug genotoxicity tests on lymphoblastoid cell lines using the Merck FLowSight® has been developed using DRAQ5™ (Verma, 2018).

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A complete list of recent papers with a brief technical review in high content screening/ drug discovery using DRAQ5™ can be found here: https://biostatusblog.blogspot.com/2018/11/DRAQ5™DDrefs2017-2018.html

Further reading:

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Technical datasheet and other key documents can be found on the DRAQ5™ product page.
You can read independent product reviews on DRAQ5™, moderated by SelectScience.

If you would like to know more about DRAQ5™ or any BioStatus product get in touch..

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