Simplified time-lapse microscopy: using differential nuclear fluorescence to track viability during cytotoxic treatment

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Testing drug treatments *in vitro* is a key aspect of many preclinical models in medical research. Predominantly, single or serial end-point metabolic assays are used to determine efficacy of a drug by inferred cell viability. However, traditional endpoint viability assays are not easily able to determine time dependent effects of treatment on cell division and cell death. Thus, alternative time-lapse monitoring options were investigated. We used a new live-cell compatible far-red DNA dye and captured images at regular intervals over 48h-72h with two microscopes, an incubated Zeiss Axio-Observer widefield microscope with automated stage and the PerkinElmer Opera Phenix.

Use of the non-toxic far-red DNA dye (SiR-Hoechst) was a key requirement for these studies. It is well known that the UV excitation, required to visualize Hoechst staining, is damaging to live cells. Furthermore, preliminary studies of some other available, non-blue, live-cell compatible nuclear stains showed inconsistent nuclear morphology and/or toxicity when incubated with cells for 24h or more. SiR-Hoechst on the other hand, was sufficiently distinct as to enable identification of different stages of mitosis as well as consistent nuclear distribution after 72h. Furthermore, SiR-Hoechst appears to differentially label live and dead cell populations in our cancer cell lines. This allowed for predictable segregation of live and dead cell populations in addition to whole cell count. Viable cell counts from imaged wells were closely correlated with an ATP dependent metabolic endpoint assay done post-imaging. Changes in cell counts were successfully monitored at 6h intervals through 3 population doublings with no effect observed as a result of the dye or imaging *per se*. There was however, a significant effect of the incubation environment associated with time spent in the microscope. Replicate plates with identical set up, but incubated in a traditional cell incubator, showed a significantly lower cytotoxic response compared to the imaged plate. Thus, the significant remaining hurdle is to improve the long-term environmental stability for live cell incubation in imaging.

This work illustrates that simple dye based fluorescent nuclear labeling and time-lapse imaging of cells *in vitro* is a viable alternative to endpoint metabolism assays. A prospect that is enticing for sensitive cell lines and precious primary cultures.