DeepDyeDrop: a high-throughput microscopy platform for phenotyping the response of cancer cell lines to therapeutic agents

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Project overview and goals

The accurate measurement of diverse phenotypic responses in cells treated with drugs is an integral step in the pre-clinical development of new therapeutics, in addition to providing insight into drug mechanisms of action. Dose-response studies aimed at identifying sensitive and resistant cell lines or differences among related compounds are increasingly performed on a relatively large scale with panels of genetically diverse cancer cell lines and compound libraries. To enable this level of throughput, these screens have typically been performed using surrogate measurements of population level cell viability, such as CellTiterGlo. Hits from such primary screens are then typically pursued using follow-up studies for specific readouts that are time consuming and expensive. An intermediate assay that enables sufficient throughput without compromising on resolution has the potential to be time-saving and cost-sparing in pre-clinical research, thus justifying the use of a richer assay at an earlier stage of compound screening. We present the Deep Dye Drop assay, a minimally disruptive, high-throughput, customizable microscopy-based method that can serve as a primary screening platform and provide phenotypic insight at the single cell level. In addition to cell viability, the Deep Dye Drop assay provides detailed cell cycle information enabling the detection of phenotypic subtleties and fractional responses otherwise missed if data are only considered at the population level. We apply these methods to identify differences in phenotypic responses among 42 ovarian cancer cell lines upon treatment with 40 small molecule kinase inhibitors.

Deep Dye Drop Assay

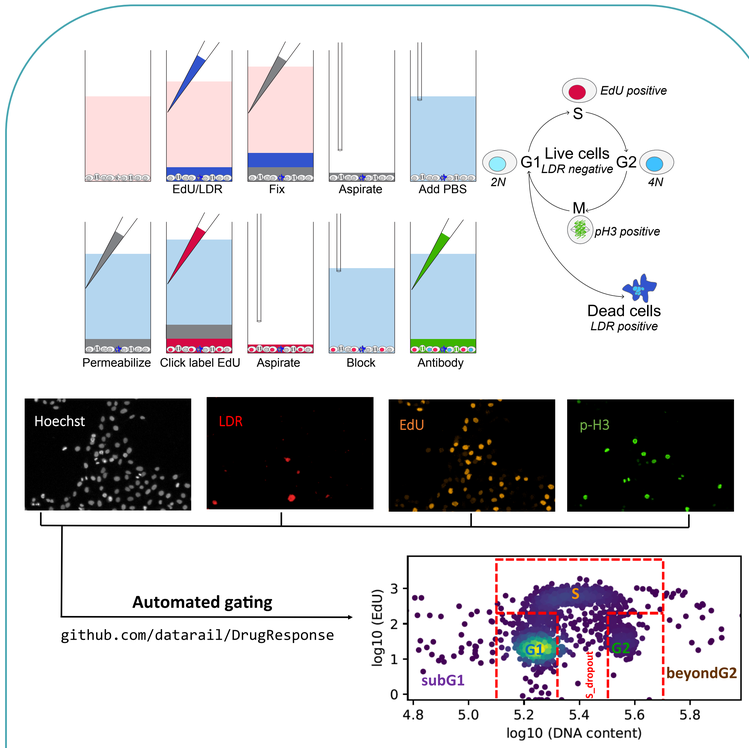

Figure 1: The Deep Dye Drop method leverages sequential density staining whereby cells adhered to plates are exposed to fixatives, wash reagents and dyes dissolved in solutions having progressively higher densities. The first solution dropped onto the cells contains the LDR dye for dead cells, and EdU for incorporation into cells in the S phase of the cell cycle. EdU is labeled with Cy3 using click chemistry. Finally, a solution containing phospho-histone H3 antibody conjugated to Alexa fluor 488 and Hoechst is used to label M-phase cells and to stain all nuclei respectively. A computational tool is applied for automated gating of single cells into different phases of the cell cycle.

Assay flexibility

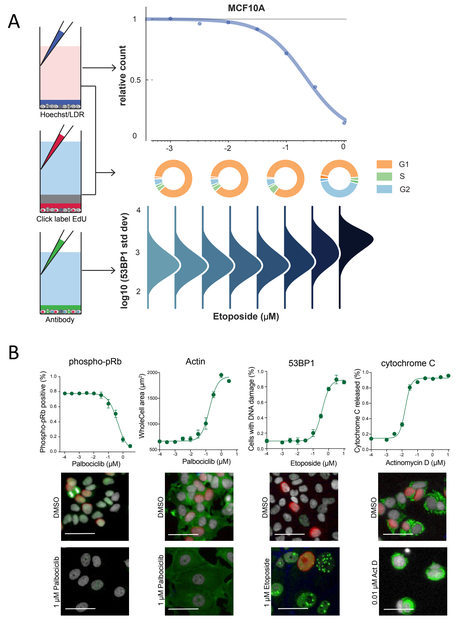

Figure 2: (A) Multiple phenotypic readouts can be measured in parallel using the Deep Dye Drop assay. The assay is flexible in that antibodies can either replace one of the 4 dyes or be included as a 5th stain to investigate markers most pertinent to the drug under study. For instance, pH3 has been swapped with a 53BP1 antibody and the single cell distribution of 53BP1 (standard deviation of intensity) is measured across a dose range of Etoposide. (B) (Left to right) Palbociclib-induced decrease in nuclear pRb phosphorylation, palbociclib-induced increase in cell size quantified using a beta-actin antibody, etoposide-induced DNA double strand breaks measured by 53BP1 foci formation, actinomycin D-induced apoptosis quantified by the nuclear to cytoplasm ratio of cytochrome C staining.

DDD enhances GR metrics

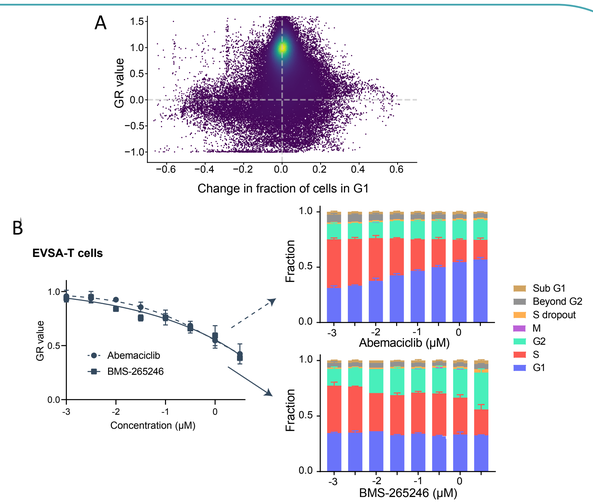

Figure 3: (A) Equivalent GR values can reflect different changes in the cell cycle distribution of the population; for example, a GR value of zero can result from loss, gain, or no change in the fraction of cells in G1 (B) EVSA-T cells treated with the CDK1/2 inhibitor BMS-265246 or the CDK4/6 inhibitor abemaciclib exhibit the same dose dependent decrease in GR values; however, the abemaciclib-treated cells arrest in G1 whereas the BMS-265426-treated cells arrest in G2.

Ovarian cancer profiling

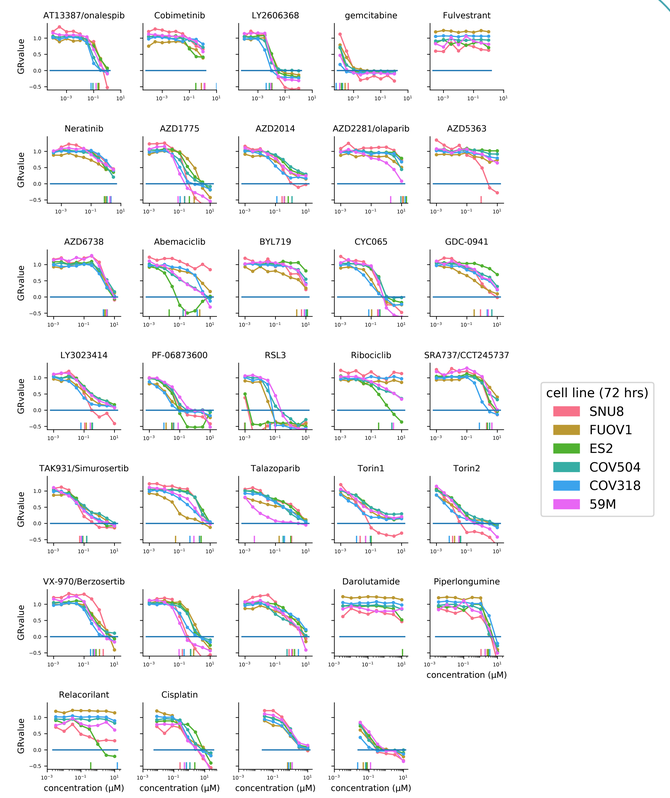

Figure 4: GR dose response curves for six ovarian cancer cell lines treated with each drug of interest.

Project status

The deep dye drop assay is robust and flexible. Antibodies can be shuffled to different channels without affecting the results, and different antibodies can be used. We have also tested a five channel deep dye drop protocol by performing dual antibody staining (primary antibodies pH3 and 53BP1, labeled with secondary antibodies Alexa 750 and Alexa 488 respectively). The assay is also compatible with high content imaging methods such as cyclic immunofluorescence (CycIF) should a user wish to build upon a deep dye drop result by further multiplexing. A manuscript describing the assay and its use in screening drugs on breast cancer cell lines is in preparation. Ovarian cancer profiling efforts (GR dose response, baseline omics, DNA damage) are ongoing.

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