Screening Cell Cycle Inhibitors Using Automated High-Content Imaging

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INTRODUCTION

Monitoring treatment effects on the cell cycle is particularly relevant to progressing oncology research and drug discovery. For instance, compounds known to inhibit mitosis are often utilized to slow division of cancer cells. Cell-based high-content assays have been developed to classify cells by cell cycle phases. Two assays will be presented for evaluating cell cycle. The first is an endpoint assay based on a single nuclear stain which may be augmented with an immunoassay for a mitotic or an apoptotic marker. The second is a 3 day live cell time-lapse assay that identifies cells using a brightfield image and then detects fluorescent protein markers that are expressed in the nuclei at defined times during cell division. We will show both approaches for cell cycle evaluation using the ImageXpress® Micro system with MetaXpress® Software. This study highlights the specific effects of cell division inhibitors Paclitaxel, Colchicine and Nocodazole on the cell cycle.

MATERIALS

- **Assay Reagents and Cells**
  HeLa cell line
  Corning® Falcon 96 well plate P/N 353219
  Premo FUCCI Cell Cycle Sensor – Life Technologies P/N P36238
  Cell cycle inhibitors – Paclitaxel, Colchicine, Nocodazole
- **High Content Imaging**
  ImageXpress® Micro High Throughput Confocal Imaging System with transmitted light and environmental control options
  MetaXpress® High-Content Image Analysis Software

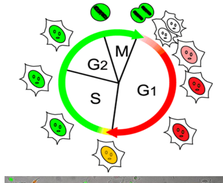


Fig 1: FUCCI Cell Cycle Reagent is a dual color sensor. Cells in G_1 express orange, cells in G_2/M express green and cells in S phase appear yellow (both red+green expressed). Brightfield images are used to also count non-fluorescent cells transitioning into G_1.

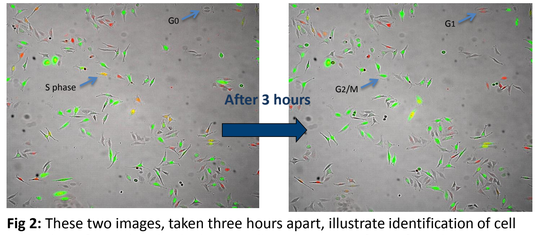


Fig 2: These two images, taken three hours apart, illustrate identification of cell cycle phase for each individual HeLa cell via detection of transfected FUCCI sensors using live-cell automated imaging.

METHODS

Add 30 particles/cell each FUCCI reagent to 40,000 cells/mL suspension of HeLa cells. Seed 4,000 cells/well of the cells into a 96 wells plate. Allow attachment in incubator at 37° C, 5 % CO2 for ~8 hours.

Treat the cells with different concentrations of cell division inhibitors and place the plate into ImageXpress Micro Confocal Imaging System equipped with environmental control.

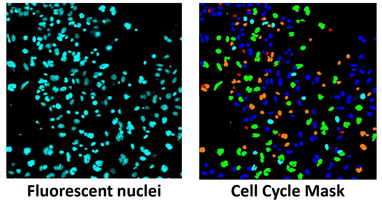
Set the instrument to image the plate every 2.5 - 3 hours in brightfield and fluorescence. Stop image acquisition after 63-88 hours (in separate time-lapse experiments).

At the end of the treatment time, fix the cells, add Hoechst nuclear stain, and acquire images of the plate at 10X or 20X magnification (endpoint assay).

Analyze time-lapse images with a custom software module and endpoint images using the Cell Cycle module in MetaXpress software.

Create movies from the images captured by the instrument during a 63 - 88 hour time frame.

ENDPOINT ASSAY UTILIZES A SIMPLE NUCLEAR STAIN



Fluorescent nuclei | Cell Cycle Mask

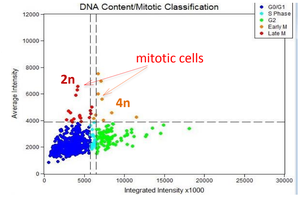


Fig 3: Configure software module to identify phases of the cell cycle. A plot of nuclear intensities will appear with interactive dashed lines (gates). Nuclei above the horizontal line will be detected as mitotic cells, which are either 4N (Early Mitotic) or 2N (Late Mitotic).

ENDPOINT ASSAY RESULTS

After selecting an image of stained nuclei and defining the size and intensity thresholds, the software settings are complete for mitotic classification. The analysis proceeds automatically on all wells and all plates specified.

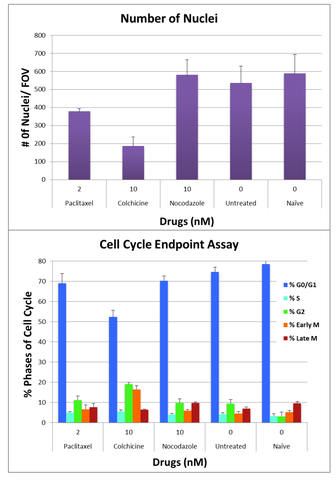
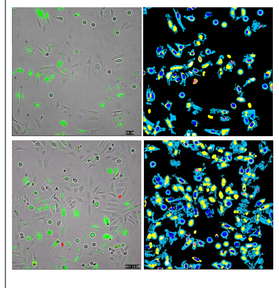


Fig 4: (Top) Total nuclei counted at the end of the time-course. Colchicine and Paclitaxel inhibited cell division compared to the untreated control. We noted no significant difference in cell count in the FUCCI-infected cells vs. naïve HeLa cells. **(Bottom)** The percentage of cells identified in each phase of the cell cycle using a nuclear stain only. More cells were classified in S phase and G_2 phase if treated with Paclitaxel or Colchicine vs. untreated controls.

LABEL-FREE IDENTIFICATION OF CELLS + FLUORESCENT CELL CYCLE CLASSIFICATION WITH FUCCI SENSOR



Mask Legend		
Layer	Color	Mask Name
1		Master Cell Mask
2		Mask to count cells
3		GFP Pos w S phase removed
4		RFP Pos w S phase removed
5		S phase nuclei

Fig 5: Time-lapse images (20X objective) of HeLa cells transfected with the FUCCI cell cycle sensor were analyzed using a MetaXpress software module that identifies cells in the transmitted light image and then scores them based on whether the nuclei are red only, green only, red+green, or non-fluorescent and classifies them as illustrated in Figure 1.

TIME-LAPSE ASSAY RESULTS

Using the FUCCI cell cycle sensor in living cells, nuclei in different phases of the cell cycle can be measured over the duration of the compound treatment rather than at a fixed endpoint only. Cells in G0, although not emitting fluorescent signal, can be counted in a transmitted light image and included in the total cell count to calculate % of cells in each phase.

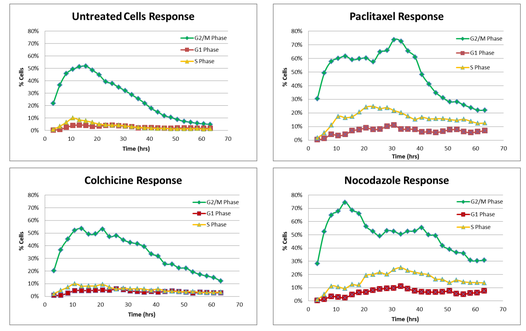


Fig. 6: Cells identified in different phases of the cell cycle at each time point over a 65 hour experiment. Paclitaxel treatment (2 nM) shows percentage of cells in S, G2 and M is significantly higher than the control. Treatment with 10 nM Colchicine shows percentage of cells in G2 and Early M is significantly higher than the control. Treatment with 100 nM Nocodazole shows a significant inhibition from dividing (also seen as no increase in cell number over the course of the experiment – data not shown).

CONCLUSIONS

- Treatment with drugs that interfere with microtubule function, thus retarding cell division, caused an increased percentage of cells arrested in the mitotic phases.
- MetaXpress software can identify and classify cells in different phases of the cell cycle using a nuclear stain and a simple endpoint image with a pre-configured software module.
- The assay may also be conducted as a time-lapse experiment to monitor cell cycle phase in living cells over hours or days and analyzed using transmitted light segmentation plus a dual fluorescent protein sensor for identifying nuclei in different phases of the cell cycle.
- Both of these assays are amenable to high throughput imaging and high content analysis.

MOLECULAR DEVICES