Automated Analysis of Apoptosis and Cytotoxicity using the IN Cell Analyzer 1000

Bohdan Soltys*, Yuriy Alexandrov, Denis Remezov, Marcin Swiatek, Samantha Murphy, Louis Dagenais and Ahmad Yekta

GE Healthcare, 500 Glenridge Ave, St. Catharines, Ontario, Canada L2S 3A1; Tel: 905.688.2040, E-mail: Bohdan.Soltys@ge.com

Abstract and Introduction

Apoptosis and toxicity testing are increasingly performed at early stages of the drug discovery process. A large number of compounds can potentially induce apoptosis or merely affect cell viability. To be potentially applicable, an algorithm must analyze major cell compartments (nucleus, cytoplasm, cell outlines) and any inclusions of any size or shape.

DNA alterations

A universal indicator of apoptosis is DNA fragmentation and granulation. Within 4 hrs of staurosporin treatment, cells show a characteristic distribution of the DNA fingerprint in nuclei and the appearance of annex V labelling on the cell surface is shown in Figure 1.

Figure 1. Segmentation. (A) DNA fragmentation/granulation (red segmentation) in the nuclear channel and (B) annex V labeling (yellow segmentation) of the cell surface in the signal channel. Blue outlines represent the nuclear edge.

Results and Discussion

Cells were assessed using a variety of early stage apoptotic assays. The apoptosis module available in the IN Cell Analyzer 1000 is a general algorithm that analyzes major cell compartments and any objects, or inclusions, within these.

Multiscale segmentation capability of this algorithm allows for simultaneous identification and quantification of subcellular inclusions of any size or shape.

DNA alterations

DNA fragmentation and granulation are key indicators of apoptosis. The appearance of annex V labelling on the cell surface is shown in Figure 1.

Cytology and false positives

Many cell-based assays are liable to false positive results due to cytotoxic effects of added compounds. It is desirable to remove from analysis entire wells or the fraction of cell population thus affected. The resulting cell rounding causes erroneous measures of intensity and morphology.

Methods

Cyclin B1–GFP and annex V labelling on the cell surface is shown in Figure 1.

Figure 1B shows the granular nature of the annexin signal. Quantitation of the binding is shown in Figure 3 for cells treated with 5 µM staurosporin for 4 hrs. The nuclear/cytoplasmic intensity ratio is the main measure, which is much higher for rounded cells as seen in Figs. 7 and 8.

References

(1) Bohdan Soltys, Yuriy Alexandrov, Denis Remezov, Marcin Swiatek, Samantha Murphy and Ahmad Yekta. 2004. Learning Algorithms Applied to Cell Subpopulation Analysis in High Content Screening. Conference of the Society of Biomolecular Screening, Orlando, Florida

Figure 7. Classification of rounded or cytotoxic cells. Cyclin B1–GFP labelling cells with (A) control (green) and (B) Segmented image showing the categorization of cells by the classification software. Normal cells, green; rounded, red.

Figure 8. Effect of rounded or control cells on the determination of nuclear/cytoplasmic intensity ratios in a nuclear translocation assay. (n=12 replicates)

Conclusions

• A generalized algorithm for analysis of a wide variety of apoptosis assays is described

• Applications demonstrated include early time point apoptotic events involving alterations to DNA, mitochondria, annexin V binding, cell morphology and aggregation states

• Learning algorithm methods and filters have been developed for increasing data quality and removing false positives resulting from cytotoxic compounds.

Figure 6. Cell morphology and inter-cell adjacency. Cells of Figs. 4 and 5. Control vs. drug-treated (4 hrs staurosporin, 5 µM). Per cell measures averaged over replicate wells. (n=10 replicates per condition)

Figure 8 shows the results of the clustering status of the cell populations of Figs. 4 and 5. The results indicate cells became more spaced out upon drug treatment, reflected in a decrease in cell neighbour count and more heterogeneous inter-cellular spacing (larger SDs). As clustering/aggregation of cells in the cell population was maximal (see Figure 4), the change in SD here is a sensitive indicator of the population status.

With respect to single cell morphology, the spreading of cytoplasm fluorescence intensity decreases upon drug treatment (Fig 6), indicating cytoplasm becomes concentrated around the nucleus. The rise in cell form factor on the other hand reflects blebbing of the cell surface (cf. cell outlines in Fig. 4B). Cells also become less spherical (elongation parameter decreases).

Cytotaxis

Many cell-based assays are liable to false positive results due to cytotoxic effects of added compounds. It is desirable to remove from analysis entire wells or the fraction of cell population thus affected. The resulting cell rounding causes erroneous measures of intensity and morphology.

Methods

Learning algorithm methods and filters have been developed for increasing data quality and removing false positives resulting from cytotoxic compounds.

References

(1) Bohdan Soltys, Yuriy Alexandrov, Denis Remezov, Marcin Swiatek, Samantha Murphy and Ahmad Yekta. 2004. Learning Algorithms Applied to Cell Subpopulation Analysis in High Content Screening. Conference of the Society of Biomolecular Screening, Orlando, Florida

Conclusions

• A generalized algorithm for analysis of a wide variety of apoptosis assays is described

• Applications demonstrated include early time point apoptotic events involving alterations to DNA, mitochondria, annexin V binding, cell morphology and aggregation states

• Learning algorithm methods and filters have been developed for increasing data quality and removing false positives resulting from cytotoxic compounds.

Figure 6. Cell morphology and inter-cell adjacency. Cells of Figs. 4 and 5. Control vs. drug-treated (4 hrs staurosporin, 5 µM). Per cell measures averaged over replicate wells. (n=10 replicates per condition)

Conclusions

• A generalized algorithm for analysis of a wide variety of apoptosis assays is described

• Applications demonstrated include early time point apoptotic events involving alterations to DNA, mitochondria, annexin V binding, cell morphology and aggregation states

• Learning algorithm methods and filters have been developed for increasing data quality and removing false positives resulting from cytotoxic compounds.

References

(1) Bohdan Soltys, Yuriy Alexandrov, Denis Remezov, Marcin Swiatek, Samantha Murphy and Ahmad Yekta. 2004. Learning Algorithms Applied to Cell Subpopulation Analysis in High Content Screening. Conference of the Society of Biomolecular Screening, Orlando, Florida

Conclusions

• A generalized algorithm for analysis of a wide variety of apoptosis assays is described

• Applications demonstrated include early time point apoptotic events involving alterations to DNA, mitochondria, annexin V binding, cell morphology and aggregation states

• Learning algorithm methods and filters have been developed for increasing data quality and removing false positives resulting from cytotoxic compounds.

Figure 6. Cell morphology and inter-cell adjacency. Cells of Figs. 4 and 5. Control vs. drug-treated (4 hrs staurosporin, 5 µM). Per cell measures averaged over replicate wells. (n=10 replicates per condition)

Conclusions

• A generalized algorithm for analysis of a wide variety of apoptosis assays is described

• Applications demonstrated include early time point apoptotic events involving alterations to DNA, mitochondria, annexin V binding, cell morphology and aggregation states

• Learning algorithm methods and filters have been developed for increasing data quality and removing false positives resulting from cytotoxic compounds.