Abstract
Apoptosis, or programmed cell death, is involved in almost every physiological and pathological process. There are numerous processes involved in apoptosis, some in sequence and others simultaneously. Using image analysis modules of MetaXpress™, we examined separate parts of the apoptotic pathway to determine whether the responses would be differentially sensitive to the toxic effects of Staurosporine treatment.

For changes in the mitochondria we looked at translocation of Cytochrome C and Bax to and from the mitochondria. We measured nuclear morphology to evaluate disruption of the nucleus, and measured cell morphology for cytoskeletal changes we used cell morphology. Although all treatments produced similar responses and IC₅₀'s there were subtle differences between the responses that might otherwise have been missed in a single parameter analysis. These results indicate that these assays may be multiplexed for finer discrimination of apoptotic pathways.

Introduction
Cell-Based High-Content Screening provides a way of analyzing multiple properties of a sample simultaneously. Apoptosis is a complex process involving multiple biochemical and physical pathways. Under different conditions and with different cell types the magnitude and temporal aspects of each of the pathways may vary. As such, a single assay for apoptosis only reports part of the story.

Various biochemical and morphological changes occur during apoptosis. Apoptotic cells degrade their chromatin causing compacted and misshapen nuclei. Changes in the cytoskeleton lead to cell morphology changes including shrinkage of cytoplasm. Mitochondria lose their membrane potential during which time certain proteins dissociate from the mitochondria. Other biochemical changes include activation of the caspases, inversion of the plasma membrane and increased permeability of plasma membrane.

Our previous study (Quantitation of Apoptosis, Necrosis and Cell Death Using High Content Screening, SBS 2004) used individual assays for cell health looking at plasma membrane permeability, inversion of the plasma membrane or mitochondrial membrane potential. In this study we have multiplexed assays for cell morphology, mitochondrial content and nuclear morphology to characterize cells and compare the responses to increased concentrations of Staurosporine.

Materials and Methods
HeLa cells were labeled with Mitotracker Red (Molecular Probes) for 30 minutes at 37°C. They were then incubated with varying concentrations of Staurosporine (10 nM – 10 µM) or DMSO for 6 hours. The cells were then fixed and stained with DAPI (nuclei) and anti-cytochrome C or anti-Bax, followed with a FITC-labeled secondary antibody as appropriate.

Images were acquired using an ImageXpress® 5000A with DAPI, FITC, and Rhodamine filter sets and a 20x Plan Fluor ELWD objective. Images were imported into the MDCStore database and analyzed using MetaXpress automated image acquisition and analysis software using a combination of turnkey application modules (Translocation Enhanced, Cell Scoring) and other MetaXpress analysis functions. Curve-fit analyses and Z' calculations were performed using AcuityXpress™ cellular informatics software.

Conclusion
Although cell morphology, nuclear morphology and translocation of proteins from the nucleus measure different aspects of the apoptotic pathways, they can all be measured simultaneously on a single sample.

All three types of assays produced results with good Z' values and comparable IC₅₀ values. Some measurement parameters displayed a more complex dose-dependent response to the staurosporine treatment. Further investigation of these phenotypes may provide insight into the mechanisms of staurosporine-mediated apoptosis.

References