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# High-Content Screening: A New Approach to Easing Key Bottlenecks in the Drug Discovery Process

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## ABSTRACT

**Recent improvements in target discovery and high throughput screening (HTS) have increased the pressure at key points along the drug discovery pipeline. High-content screening (HCS) was developed to ease bottlenecks that have formed at target validation and lead optimization points in the pipeline. HCS defines the role of targets in cell functions by combining fluorescence-based reagents with the ArrayScan™ System to automatically extract temporal and spatial information about target activities within cells. The ArrayScan System is a tabletop instrument that includes optics for subcellular resolution of fluorescence signals from many cells in a field within a well of a microtiter plate. One demonstrated application is a high-content screen designed to measure the drug-induced transport of a green fluorescent protein—human glucocorticoid receptor chimeric protein from the cytoplasm to the nucleus of human tumor cells. A high-content screen is also described for the multiparametric measurement of apoptosis. This single screen provides measurements of nuclear size and shape changes, nuclear DNA content, mitochondrial potential, and actin–cytoskeletal rearrangements during drug-induced programmed cell death. The next generation HCS system is a miniaturized screening platform, the CellChip™ System, that will increase the throughput of HCS, while integrating HCS with HTS on the same platform.**

## INTRODUCTION

THE EARLY STAGES OF THE DRUG DISCOVERY PROCESS form a continuous pipeline of steps including target identification, target selection, target validation, primary screening, and candidate optimization (Fig. 1). A major challenge is to improve the productivity of identifying lead compounds while traversing this pipeline. Productivity has been improved in target identification and selection through the development of automated DNA sequencing and genomics databases. Target validation is now a key bottleneck because time-consuming biological assays are usually performed without automation. The plethora of new genes identified by genomics has also increased the pressure at this point in the pipeline. Primary screening has been improved over the last decade due to the implementation of automated, high throughput screening (HTS) systems. However, there is now a new bottleneck in primary screening caused by the dramatic increase in the size of compound libraries generated primarily by combinatorial chemistry. Therefore, new technologies are required to increase the throughput. There continues to be a bottleneck in candidate optimization that will get worse with increased numbers of hits generated by new, higher throughput screening systems. Furthermore, secondary screens, structure–activity relationship (SAR) studies and cytotoxicity assays are not as automated as primary screens in most pharmaceutical companies. It is possible to improve the productivity substantially by developing new fluorescence-based, automated approaches to target validation and candidate optimization using living cells analyzed in space and time.[1,2]

There are three major driving forces involved in improving the productivity of the early drug discovery process. First, there is a need for increased information-handling tools. Bioinformatics has blossomed with the rapid development of DNA-sequencing systems and the evolution of the genomics database. Genomics is beginning to play a critical role in the identification of potential new targets. Proteomics has become indispensible in relating structure and function of protein targets to predict drug interactions. The next level of biological complexity is the cell. Therefore, there is a need to acquire, manage, and search multi-dimensional information from cells. Second, there is a need for higher throughput tools. Automation is a key to improving productivity as has already been demon-

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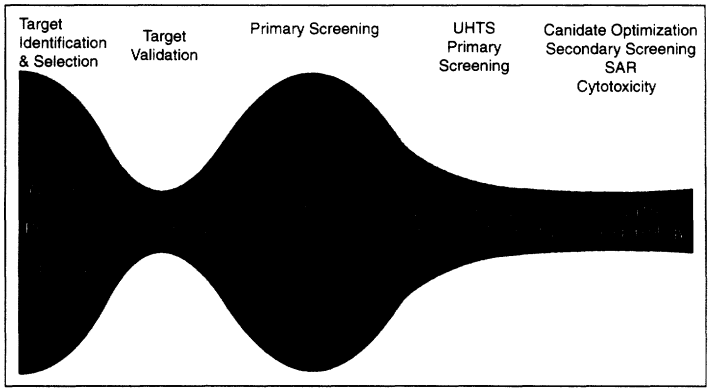


**FIG. 1.** Some key bottlenecks in the early drug discovery process. The pipeline depicts the current technologies or knowledge that maximally impacts each segment of the drug discovery process. Constrictions have occurred at sites of target validation, ultra-high throughput screening (HTS), and candidate optimization due to a lack of appropriate technology and automation. High-content screening (HCS) can deliver solutions to the target validation and candidate optimization bottlenecks today by combining a live cell screening format with fluorescence-based molecular reagents and computer-based feature extraction, data analysis, and automation. The result is better decisions, shortened cycle times, and ultimately, faster evaluation of promising drug candidates.

strated in DNA sequencing and high throughput primary screening. Automated systems to extract multiple parameter information from cells is now required. Third, there is a need to miniaturize the methods. Miniaturization will allow increased throughput while decreasing the volumes of reagents and test compounds required in each assay.

The three driving forces just described have led to a paradigm shift in the early drug discovery process. Radioactivity has been the dominant read-out in assays. However, the need for more information, higher throughput, and miniaturization has caused a shift toward using fluorescence detection. Fluorescence-based reagents will yield more powerful, multiple parameter assays that will be higher in throughput and information content and will require lower volumes of reagents and test compounds (Fig. 2). Fluorescence is also safer and less expensive than radioactivity-based methods. The use of isolated tar-

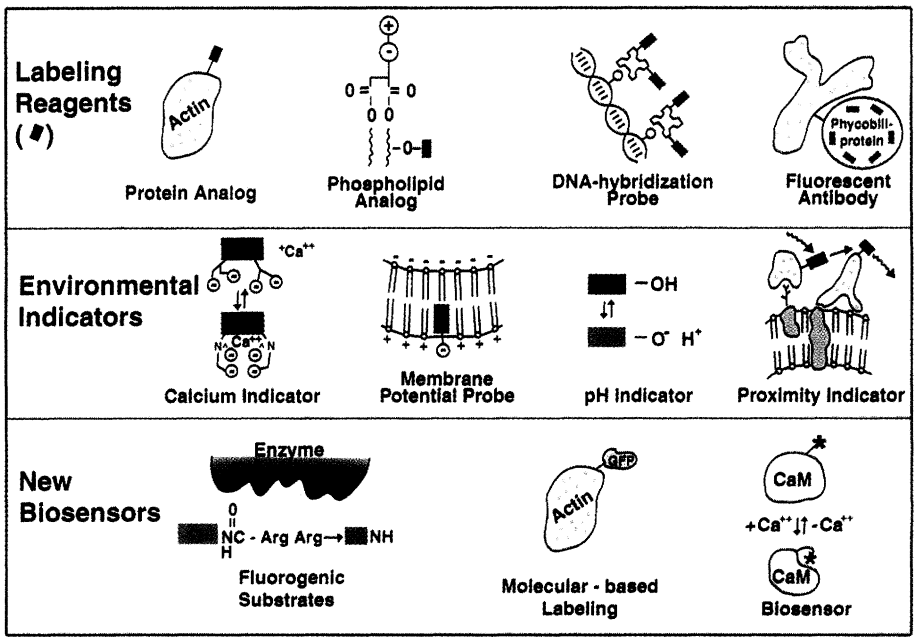


**FIG. 2.** Fluorescent reagents for drug discovery. A major component of the new drug discovery paradigm is a continually growing family of fluorescent reagents that are used to measure the temporal and spatial distribution, content, and activity of intracellular ions, metabolites, macromolecules, and organelles. Classes of these reagents include labeling reagents that measure the distribution and amount of molecules in living and fixed cells, environmental indicators to report signal transduction events in time and space, and fluorescent protein biosensors to measure target molecular activities within living cells. A multiparameter approach that combines several reagents in a single cell is a powerful new tool for drug discovery. The new classes of biosensors are key BioDx technologies that are being developed.

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gets has also been the major format for screening. However, there is more information in assaying specific targets in the natural environment of the cell. Therefore, more cell-based assays will be developed. The combination of multicolor fluorescence detection applied to living cells will yield a wealth of critical information on the function of specific target molecules and cellular processes in cell functions.[1,3,4]

### *High-content screening (HCS)*

Cells are the basic units of life and thus perform all life functions. Cells integrate the information from DNA, RNA, proteins, metabolites, and ions, and encode the normal and abnormal traits of life. The temporal–spatial interplay of ions, metabolites, macromolecules, and organelles are responsible for performing the complex reactions that perform the life functions. It has become evident in the last few years that cell functions involve changes in the subcellular distribution of cellular constituents in addition to their activity. Therefore, a deep understanding of the role of selected targets in cell functions requires tools to automatically extract temporal and spatial information about target activities. In addition, a variety of cell processes such as endocytosis, exocytosis, and intracellular transport can be assayed without initially knowing the specific "target" molecules involved.

High-content screens have been developed to address the need for more detailed information about the temporal–spatial dynamics of cell constituents and processes. High-content screens automate the extraction of multicolor fluorescence information derived from specific fluorescence-based reagents incorporated into cells[2,5] (Fig. 2). Cells are analyzed using an optical system that can measure spatial as well as temporal dynamics.[1,6–8] The concept is to treat each cell as a "well" that has spatial and temporal information on the activities of the labeled constituents. High-content screens will yield information that will permit more efficient lead optimization before investing in the time-consuming and expensive animal-testing stage (Fig. 3).

There is a large and growing list of known biochemical and molecular processes in cells that involve translocations and/or reorganizations of specific molecules and organelles within the cells, in addition to specific biochemical changes such as phosphorylation. The binding of ligands to receptors usually results in the internalization of the receptor into the cytoplasm followed by a variety of targeting routes within the cell. In response to stimulation, some cytoplasmic components are recruited to the plasma membrane. For example, it is known that the GTP-binding proteins of the Rho family are maintained as cytoplasmic complexes with RhoGDI in resting cells but translocate to the plasma membrane during activation.[9] Furthermore, many cytoplasmic proteins, such as the glucocorticoid receptor, translocate into the nucleus as part of the signaling pathway.[10] In addition, specific organelles such as the cytoskeleton, nuclear envelope, chromatin, Golgi apparatus, mitochondria, and endosomes are reorganized in response to specific stimuli. The temporal and spatial control of the assembly and disassembly of organelles, such as the mitotic spindle, are also intimately involved in specific cell functions. Defining the role of specific "targets" in cell functions requires HCS to yield the temporal–spatial dynamic information required for a detailed understanding of the role of selected targets in cell functions and to determine the specificity of lead compounds as part of the drug discovery process.

The types of biochemical and molecular information now accessible through fluorescence-based reagents applied to cells include ion concentrations, membrane potential, specific translocations, enzyme activities, gene expression, as well as the presence, amounts, and patterns of metabolites, proteins, lipids, carbohydrates, and nucleic acid sequences. The availability of existing reagents together with the rapid development of new classes of fluorescence-based reagents, offers an extraordinary opportunity to create automated, high-content assays of cells.[4,5,11]

There are two types of high-content screens that can be performed: (a) fixed cells using fluorescently labeled antibodies, biological ligands, and/or nucleic acid hybridization probes; and (b) live cells using multicolor fluorescent indicators and "biosensors" (Fig. 2). The choice of fixed versus live cell screens depends on the specific cell-based assay required.

Fixed cell assays are the simplest, because an array of initially living cells in a microtiter plate format can be treated with various compounds and doses being tested, then the cells can be fixed, labeled with specific reagents, and measured. No environmental control of the cells is required after fixation, spatial information is acquired, but only one time point is possible. The availability of thousands of antibodies, ligands, and nucleic acid hybridization probes that can be applied to cells makes this an attractive approach for many types of cell-based screens. The fixation and labeling steps can be automated, allowing efficient processing of assays. Multicolor fluorescence assays easily permit four distinct reagents to be analyzed in each cell.

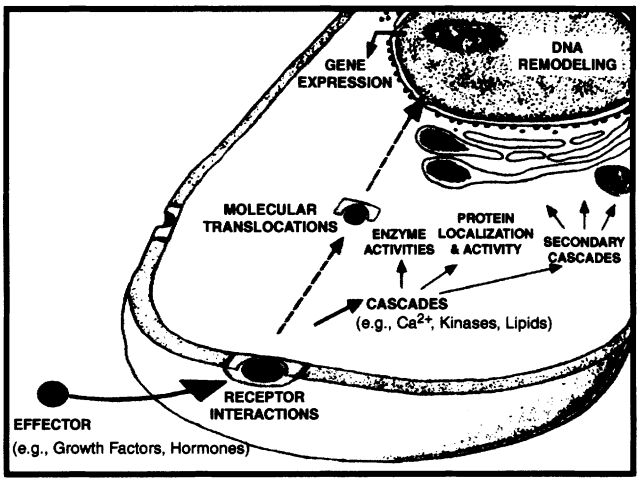


**FIG. 3.** High-content cell screening (HCS). HCS can be used to measure the effects of drugs on complex molecular events such as signal transduction pathways, as well as effects on cell functions like apoptosis, division, cell adhesion, locomotion, exocytosis, and cell–cell communication. This diagram demonstrates some of the complex temporal and spatial changes in activities that are involved in cellular processes. Multicolor fluorescence permits multiple targets and cell processes to be assayed in a single screen. Cross-correlation of cellular responses will yield a wealth of information required for target validation and lead optimization.

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Live cell assays are the most sophisticated and powerful because an array of living cells contains the desired reagents and can be screened over time as well as space. Environmental control of the cells (temperature, humidity, and carbon dioxide) is required during measurement, because the physiological health of the cells must be maintained for multiple fluorescence measurements over time. There is a growing list of fluorescent, physiological indicators, and "biosensors" that can report changes in biochemical and molecular activities within cells.[5,12]

The availability and use of fluorescence-based reagents has helped to advance the development of both fixed and live cell, high-content screens. Advances in instrumentation to automatically extract multicolor, high-content information has recently made it possible to develop HCS into an automated tool. A combination of the biological heterogeneity of cells in populations,[13,14] as well as the high spatial and temporal frequency of chemical and molecular information present within cells, makes it impossible to extract high-content information from populations of cells using existing whole microtiter plate readers. The ArrayScan™ system is the first HCS platform designed for multicolor, fluorescence-based screens using cells that are analyzed individually.

### *The ArrayScan™ System*

The ArrayScan System is a fully automated platform for high-content screens performed in microtiter plates (Fig. 4). It supports multicolor fluorescence assays with up to four channels of fluorescence. Therefore, multiparameter assays are possible. The ArrayScan System is a tabletop instrument that includes optics that permit subcellular resolution of fluorescence signals from many cells in a field within a well of a microtiter plate. In addition, the system contains microtiter plate scanning hardware, fluorescence excitation, and emission optics, a solid-state camera, a Pentium-based PC with powerful processing, analyses, and database management capabilities. An option is available to control the temperature, humidity, and gas for time series of live cell assays. The ArrayScan System automatically scans a microtiter plate acquiring multicolor fluorescence image datasets of fields of cells at a pre-selected spatial resolution. Most HCS assays can be performed with a spatial resolution of 0.68 $\mu$m, thus allowing for subcellular measurements. Morphometric information can be included in the screens because cell and organelle shapes and sizes can also be measured. Data can be presented on a cell-by-cell basis, as averages of wells or simply as "hit" reports.

Specific classes of cell-based screens and individual screens within a class are developed around the ArrayScan platform by integrating the specific cells (primary or cultured cell lines), fluorescence-based reagents (Fig. 2) and sophisticated algorithms to form a screen (Fig. 5). Several classes of screens have already been developed and a complete portfolio of distinct classes of screens will become available this year. A complete ArrayScan System includes the instrument, a bioinformatics workstation, cells, reagents, specific software, screens, and screen development support. Two screens are presented here as examples.

BioDx, Inc., supplies all components as part of a co-marketing, sales and service alliance with Carl Zeiss Jena GmbH. BioDx and Zeiss are developing a range of tools, starting with the ArrayScan System, to support the early drug discovery process. The optical and automation expertise of Zeiss with a world-wide marketing, sales, and service capability is being combined with the fluorescence-based reagent, cell screening, imaging, and informatics expertise of BioDx to serve the drug-discovery market.

## MATERIALS AND METHODS

### *High-content screen of human glucocorticoid receptor translocation*

*Plasmid construct.* A eukaryotic expression plasmid containing a coding sequence for a green fluorescent protein–human glucocorticoid receptor (GFP–hGR) chimera was prepared using GFP mutants.[15] The construct was used to transfect a human cervical carcinoma cell line (HeLa).

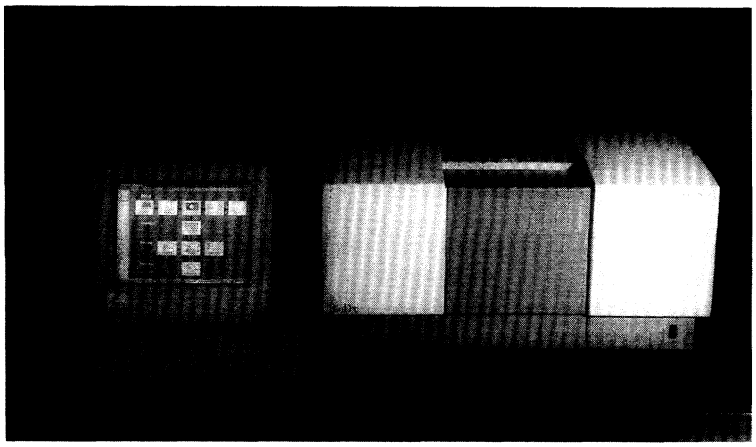

**FIG. 4.** The ArrayScan System. Shown here is the instrumentation component of the system that analyzes microtiter plates with the standard footprint. Other components of the system include the high-content screens, consisting of the reagents and algorithms, and the MultiView™ database management system.

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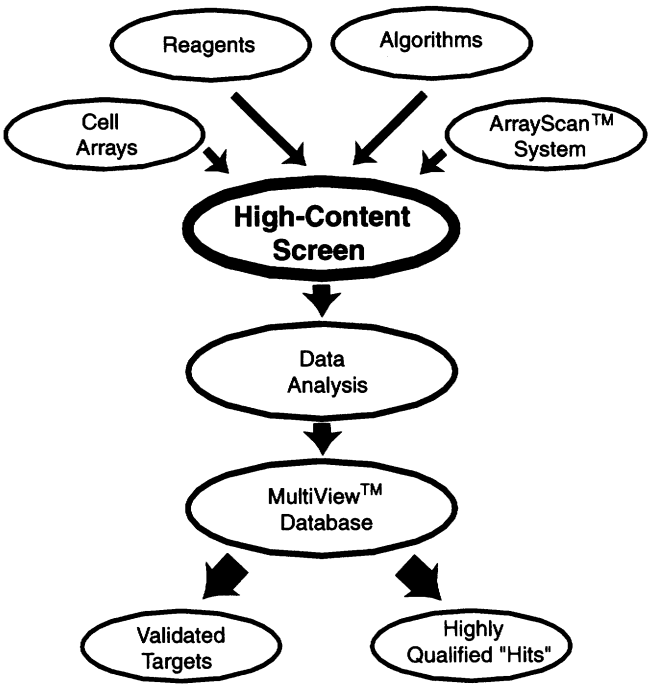


**FIG. 5.** Components of HCS. A high-content screen is the integration of arrays of live cells (primary or established cell lines), fluorescence-based reagents, feature extraction algorithms, and the ArrayScan System instrumentation including robotics. The MultiView™ database, using data obtained from HCS as input, provides data management capabilities to validate potential targets and qualify lead compounds for further optimization.

*Cell preparation and transfection.* HeLa cells were trypsinized and plated using DMEM containing 5% charcoal/dextran-treated fetal bovine serum (FBS) (HyClone, Logan, UT) and 1% penicillin-streptomycin (C-DMEM) 12–24 h prior to transfection and incubated at 37°C and 5% $CO_2$. Transfections were performed by calcium phosphate coprecipitation[16,17] or with Lipofectamine (Life Technologies Gaithersburg, MD). For the calcium phosphate transfections, the medium was replaced, prior to transfection, with DMEM containing 5% charcoal/dextran-treated FBS. Cells were incubated with the calcium phosphate–DNA precipitate for 4–5 h at 37°C and 5% $CO_2$, washed 3–4 times with DMEM to remove the precipitate, followed by the addition of C-DMEM.

Lipofectamine transfections were performed in serum-free DMEM without antibiotics according to the manufacturer's instructions. Following a 2–3 h incubation with the DNA–liposome complexes, the medium was removed and replaced with C-DMEM. All transfected cells in 96-well microtiter plates were incubated at 33°C and 5% $CO_2$ for 24–48 h prior to drug treatment. Experiments were performed with the receptor expressed transiently in HeLa cells.

*Dexamethasone induction of GFP–hGR translocation.* To obtain receptor–ligand translocation kinetic data, nuclei of transfected cells were first labeled with 5 $\mu$g/ml Hoechst 33342 (Molecular Probes, Eugene, OR) in C-DMEM for 20 min at 33°C and 5% $CO_2$. Cells were washed once in Hank's Balanced Salt Solution (HBSS) followed by the addition of 100 nM dexamethasone in HBSS with 1% charcoal/dextran-treated FBS. To obtain fixed time point dexamethasone titration data, transfected HeLa cells were first washed with DMEM and then incubated at 33°C and 5% $CO_2$ for 1 h in the presence of 0–1000 nM dexamethasone in DMEM containing 1% charcoal/dextran-treated FBS. Cells were analyzed live or they were rinsed with HBSS, fixed for 15 min with 3.7% formaldehyde in HBSS, stained with Hoechst 33342, and washed before analysis. The intracellular GFP-hGR fluorescence signal was not diminished by this fixation procedure.

*Image acquisition and analysis on the ArrayScan System.* Kinetic data were collected by acquiring fluorescence image pairs (GFP-hGR and Hoechst 33342-labeled nuclei) from fields of living cells at 1-min intervals for 30 min after the addition of dexamethasone. Likewise, image pairs were obtained from each well of the fixed time-point screening plates 1 h after the addition of dexamethasone. In both cases, the image pairs obtained at each time point were used to define nuclear and cytoplasmic regions in each cell. Translocation of GFP-hGR was calculated by dividing the integrated fluorescence intensity of GFP-hGR in the nucleus by the integrated fluorescence intensity of the chimera in the cytoplasm or as a nuclear-cytoplasmic difference of GFP fluorescence. In the fixed time-point screen, this translocation ratio was calculated from data obtained from at least 200 cells at each concentration of dexamethasone tested. Drug-induced translocation of GFP-hGR from the cytoplasm to the nucleus was, therefore, correlated with an increase in the translocation ratio.

### *High-content screen of drug-induced apoptosis*

*Cell preparation.* The day before treatment with an apoptosis-inducing drug, 3,500 cells were placed into each well of a 96-well plate and incubated overnight at 37°C in a humidified 5% $CO_2$ atmosphere. The cells chosen for this study were mouse connective tissue fibroblasts (L-929; ATCC CCL-1) and a highly invasive glioblastoma cell line (SNB-19).[18] The next day, the culture medium was removed from each well and replaced with fresh medium containing various concentrations of paclitaxel (0–50 $\mu$M) from a 20-mM stock made in DMSO. The maximal concentration of DMSO used in these experiments was 0.25%. The cells were then incubated for 26 h as just described. At the end of the paclitaxel treatment period, each well received fresh medium containing 750 nM Mito Tracker Red (Molecular Probes) and 3 $\mu$g/ml Hoechst 33342 DNA-binding dye (Molecular Probes) and was incubated for 20 min. Each well on the plate was then washed with HBSS and fixed with 3.7% formaldehyde in HBSS for 15 min at room temperature. The formaldehyde was washed out with HBSS and the cells were permeabilized for 90 s with 0.5% (v/v) Triton X-100, washed with HBSS, incubated with 2 U $ml^{-1}$ Bodipy FL phallacidin (Molecular Probes) for 30 min, and washed with HBSS. The wells on the plate were then filled with 200 $\mu$l HBSS, sealed, and the plate stored at 4°C if necessary. The fluorescence signals from plates stored this way were stable for at least 2 weeks after preparation. As in the nuclear translocation assay, fluorescence reagents can be designed to convert this assay into a live cell high-content screen.

*Image acquisition and analysis on the ArrayScan System.* The fluorescence intensity of intracellular Mito Tracker Red,

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Hoechst 33342, and Bodipy FL phallacidin was measured with the ArrayScan System. Morphometric data from each pair of images obtained from each well were also obtained to detect each object in the image field (e.g., cells and nuclei), and to calculate its size, shape, and integrated intensity.

*Calculations and output.* A total of 50–250 cells were measured per image field. For each field of cells, the following calculations were performed: (a) The average nuclear area ($\mu m^2$) was calculated by dividing the total nuclear area in a field by the number of nuclei detected; (b) The average nuclear perimeter ($\mu m$) was calculated by dividing the sum of the perimeters of all nuclei in a field by the number of nuclei detected in that field. Highly convoluted apoptotic nuclei had the largest nuclear perimeter values; (c) The average nuclear brightness was calculated by dividing the integrated intensity of the entire field of nuclei by the number if nuclei in that field. An increase in nuclear brightness was correlated with increased DNA content; (d) The average cellular brightness was calculated by dividing the integrated intensity of an entire field of cells stained with MitoTracker dye by the number of nuclei in that field. Because the amount of MitoTracker dye that accumulates within the mitochondria is proportional to the mitochondrial potential, an increase in the average cell brightness is consistent with an increase in mitochondrial potential; (e) The average cellular brightness was also calculated by dividing the integrated intensity of an entire field of cells stained with Bodipy FL phallacidin dye by the number of nuclei in that field. Because the phallotoxins bind with high affinity to the polymerized form of actin, the amount of Bodipy FL phallacidin dye that accumulates within the cell is proportional to actin polymerization state. An increase in the average cell brightness is consistent with an increase in actin polymerization.

## RESULTS AND DISCUSSION

### *High-content screen of drug-induced GFP-hGR translocation*

One class of HCS involves the drug-induced dynamic redistribution of intracellular constituents. The human glucocorticoid receptor (hGR), a single "sensor" in the complex environmental response machinery of the cell, binds steroid molecules that have diffused into the cell. The ligand–receptor complex translocates to the nucleus where transcriptional activation occurs.[10] In general, hormone receptors are excellent drug targets because their activity lies at the apex of key intracellular signaling pathways. Therefore, a high-content screen of hGR translocation has distinct advantage over *in vitro* ligand–receptor binding assays. The availability of up to two more channels of fluorescence in the ArrayScan System permits the screen to contain two additional parameters in parallel, such as other receptors, other distinct targets or cellular processes.

The design of a high-content screen for drug-induced hGR translocation is straightforward: (a) Indicator cells, human HeLa cells in this case, are cultured in a 96-well plate and are transiently transfected with a plasmid coding for a GFP-hGR chimeric protein; (b) The indicator cells are then treated with lead compounds and the translocation of GFP-hGR into the nucleus is measured either over time or at a fixed time point. The parameters of the screen are shown schematically in Fig. 6. Although a stably transfected cell line would yield the most consistently labeled cells, the ArrayScan system allows the measurement of individual cells over a wide range of signal.

To execute the screen, the ArrayScan System scans each well of the plate, images a population of cells in each, and analyzes cells individually. Here, two channels of fluorescence are used to define the cytoplasmic and nuclear distribution of the GFP-hGR within each cell. Depicted in Fig. 7 is the graphical user interface of the ArrayScan System during HCS. Because the ArrayScan System analyzes image data as it is being collected, immediate feedback about potential lead compounds is available (Fig. 7). There is a link between the image database and the information database that is a powerful tool during the validation process of new screens. At the completion of a screen, the user has total access to image and calculated data (Fig. 8).

A powerful aspect of HCS with the ArryScan System is the capability of kinetic measurements using multicolor fluorescence and morphometric parameters in living cells. Temporal and spatial measurements can be made on single cells within a population of cells in a field. Figure 9 shows kinetic data for the dexamethasone-induced translocation of GFP-hGR in several cells within a single field. It is important to note that the

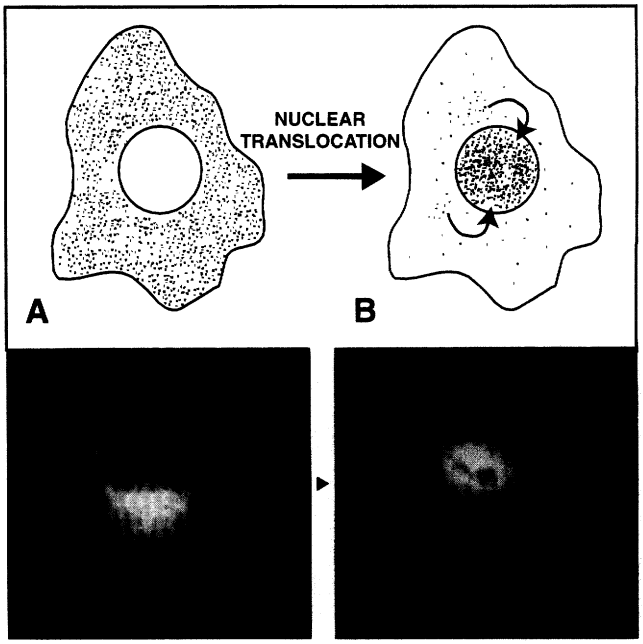


**FIG. 6.** Measurement of the drug-induced cytoplasm to nuclear translocation of the human glucocorticoid receptor. The upper pair of schematic diagrams depicts the localization of GFP-hGR within the cell before (A) and after (B) stimulation with dexamethasone. Under these experimental conditions, the drug induces a large portion of the cytoplasmic GFP-hGR to translocate into the nucleus. This redistribution is quantified by ratioing the integrated intensities of the cytoplasmic and nuclear fluorescence in treated and untreated cells. The lower pair of fluorescence micrographs show the dynamic redistribution of GFP-hGR in a single cell. The HCS is performed on wells containing hundreds to thousands of transfected cells and the translocation is quantified for each cell in the field exhibiting GFP fluorescence.

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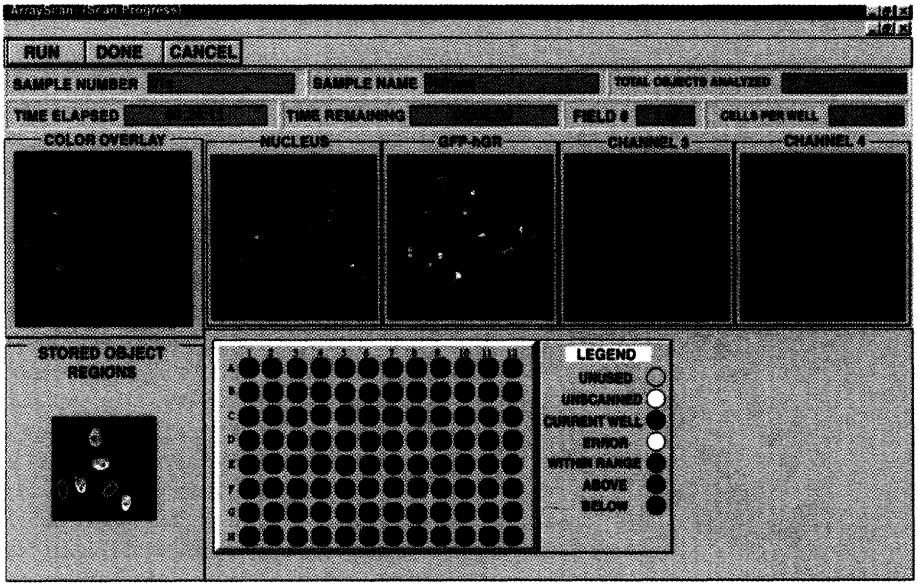


**FIG. 7.** Graphical user interface of the ArrayScan System near the end of a GFP-hGR screen. The user interface depicts the parallel data collection and analysis capability of the ArrayScan System. The windows labeled "Nucleus" and "GFP-hGR" show the pair of fluorescence images being obtained and analyzed in a single field. The window labeled "Color Overlay" is formed by pseudocoloring the above images and merging them so the user can immediately identify cellular changes. Within the "Stored Object Regions" window, an image containing each analyzed cell and its neighbors is presented as it is archived. Furthermore, as the HCS data are being collected, they are analyzed, in this case, for GFP-hGR translocation, and translated into an immediate "hit" response. The 96-well plate depicted in the lower window of the screen shows which wells have met a set of user-defined screening criteria. For example, a red-colored well indicates that the drug-induced translocation has exceeded a predetermined threshold value of 50%. On the other hand, a blue-colored well indicates that the drug being tested induced less than 10% translocation. Green-colored wells indicate "hits" where the translocation value fell between 10% and 50%. Row "E" on the 96-well plate being analyzed shows a titration with a drug known to activate GFP-hGR translocation, dexamethasone. This example screen used only two fluorescence channels. Two additional channels (Channels 3 and 4) are available for parallel analysis of other specific targets, cell processes, or cytotoxicity to create multiple parameter screens.

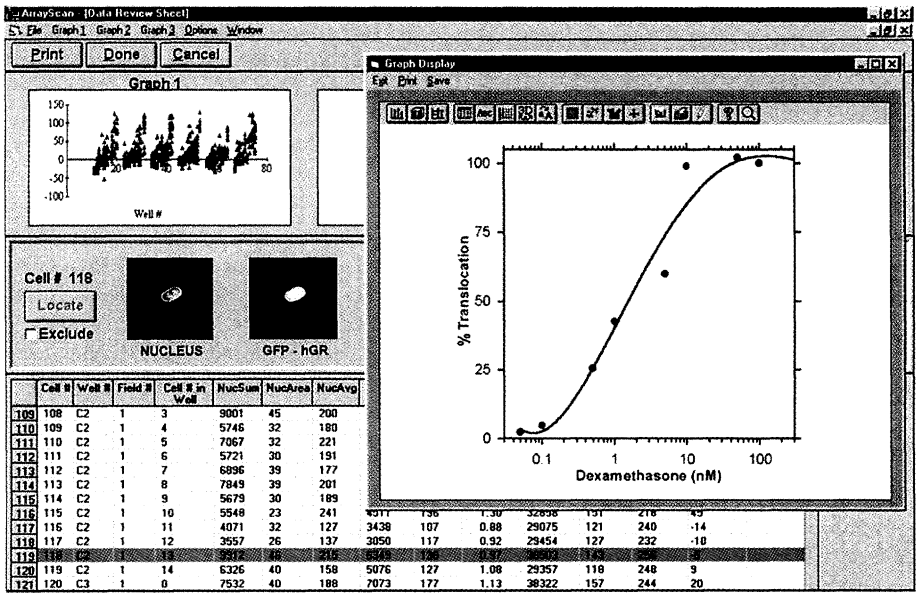


**FIG. 8.** Computer screen at end of the GFP-hGR translocation HCS with data presentation. The comprehensive data analysis package of the ArrayScan System allows the user to examine HCS data at multiple levels. Images and detailed data in a spread sheet for individual cells can be viewed separately or summary data can be plotted. For example, the calculated results of a single parameter for each cell in a 96-well plate are shown in the panel labeled Graph 1. By selecting a single point in the graph, the user can display the entire data set for a particular cell that is recalled from an existing database. Shown here are the image pair and detailed fluorescence and morphometric data from a single cell (Cell #118, magenta line). The large graphical insert shows the results of dexamethasone concentration on the translocation of GFP-hGR. Each point is the average of data from at least 200 cells. The calculated $EC_{50}$ for dexamethasone in this assay is 2 nM.

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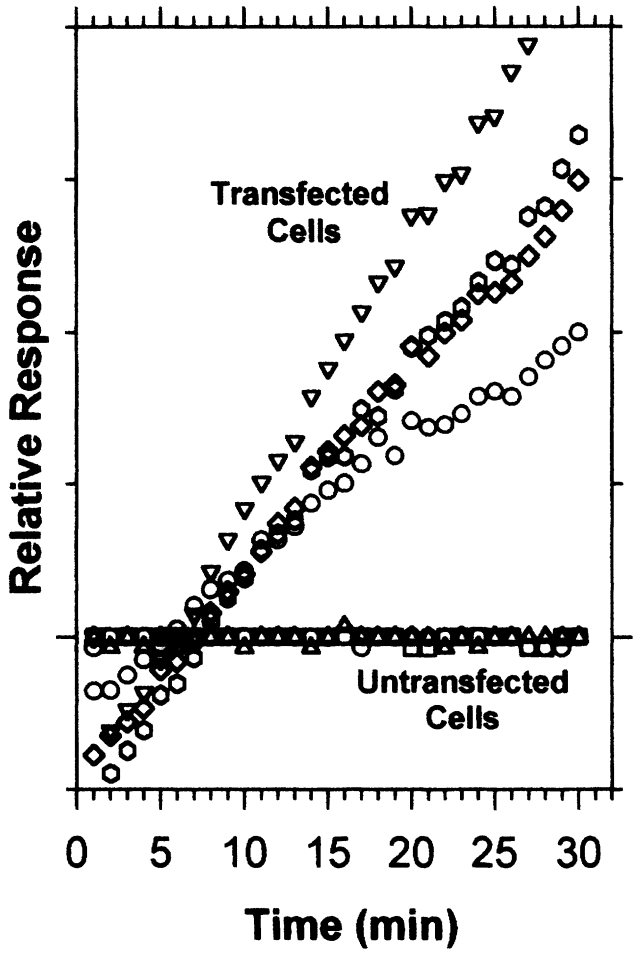


**FIG. 9.** Kinetics of GFP-hGR translocation in individual living cells in a field of cells being analyzed. Human HeLa cells transfected with GFP-hGR were treated with 100 nM dexamethasone and the translocation of GFP-hGR was measured over time in a population of single cells. The graph shows the response of transfected cells and nontransfected cells. These data also illustrate the ability to analyze cells with different expression levels.

heterogeneous levels of GFP-hGR expression induced by transient transfection did not interfere with analysis by the ArrayScan System.

*High-content screen of drug-induced apoptosis*

Apoptosis is a complex cellular program that involves myriad molecular events and pathways. To understand the mechanisms of drug action on this process, it is essential to measure as many of these events within cells as possible with temporal and spatial resolution. Therefore, an apoptosis screen that requires little cell sample preparation yet provides an automated readout of several apoptosis-related parameters would be ideal. A cell-based assay designed for the ArrayScan System has been used to simultaneously quantify several of the morphological, organellar, and macromolecular hallmarks of paclitaxel-induced apoptosis.

Two cell lines, SNB-19 and L-929, were chosen to test the high-content apoptosis assay. Cells from each line were titrated with paclitaxel, fluorescently labeled, and measured with the ArrayScan System. Figure 10 (top panels) shows the changes paclitaxel induced in one parameter, the nuclear morphology of L-929 cells. Increasing amounts of paclitaxel caused nuclei to enlarge and fragment. Quantitative analysis of these and other images obtained by the ArrayScan System is presented in the same figure. Each parameter measured showed that the L-929 cells were less sensitive to low concentrations of paclitaxel than were SNB-19 cells. At higher concentrations, though, the L-929 cells showed a response for each parameter measured. The multiparameter approach of this assay is useful in dissecting the mechanisms of drug action. For example, the area, brightness, and fragmentation of the nucleus and actin polymerization values reached a maximum value when SNB-19 cells were treated with 10 nM paclitaxel (Fig. 10; top and bottom graphs). However, mitochondrial potential was minimal at the same concentration of paclitaxel (Fig. 10; middle graph). The fact that all the parameters we measured approached control levels at increasing paclitaxel concentrations (>10 nM) suggests that SNB-19 cells have low-affinity drug metabolic or clearance pathways that are compensatory at sufficiently high levels of the drug. Contrasting the drug sensitivity of SNB-19 cells, L-929 showed a different response to paclitaxel. These fibroblastic cells showed a maximal response in many parameters at 5 $\mu$M paclitaxel, a 500-fold higher dose than SNB-19 cells. Furthermore, the L-929 cells did not show a sharp decrease in mitochondrial potential at any of the paclitaxel concentrations tested. This result is consistent with the presence of unique apoptosis pathways between a normal and cancer cell line. Therefore, these results indicate that a relatively simple fluorescence labeling protocol can be coupled with the ArrayScan System to produce a high-content screen of key events involved in programmed cell death.

*Target validation*

The application of newly discovered gene sequences to drug discovery is a key example where HCS will have an impact on target validation. In this scenario, a gene sequence coding for a potential target is first "tagged" by forming a chimera with a reporter sequence. Several possibilities for reporter sequences have been reviewed[19] and are amenable to HCS. If, for example, a target-GFP chimera were used to transfect a cell population, then HCS could be used to define the cellular location of the target and then measure drug-induced redistribution of the target within a mixed population of transfected and untransfected cells (see Fig. 9). Furthermore, the function of the potential target could be dissected by combining other fluorescence reagents (Fig. 2) with the target-GFP construct in the same living cells. If, for instance, the target-GFP construct coded for a membrane receptor, then a high-content screen could be designed to measure and correlate receptor–ligand interaction kinetics with a host of other cellular and molecular parameters within the same transfected cell (Fig. 3). HCS can, therefore, ease a major bottleneck in the drug discovery pipeline by validating the profusion of potential targets that have been created through recent advances in genomics.

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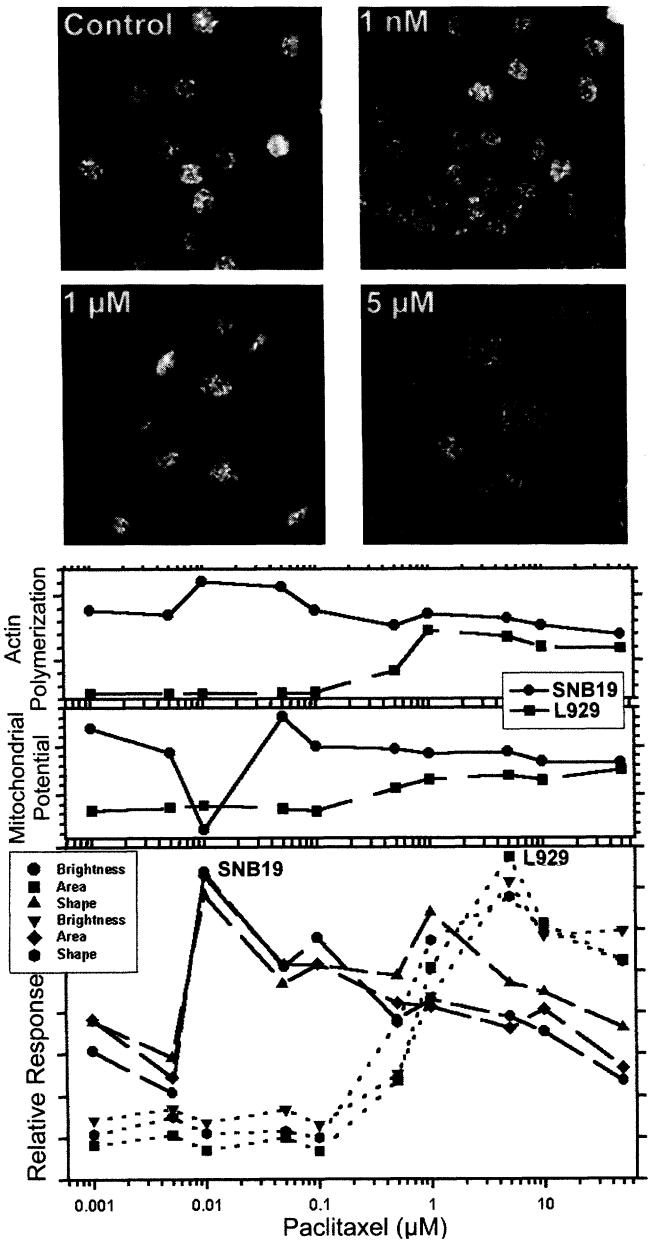


**FIG. 10.** High-content screen of drug-induced apoptosis. Normal fibroblasts (L-929) and glioblastoma (SNB-19) cells were treated with various concentrations of paclitaxel and their response was measured with a high-content screen. Shown along the top of the figure are example images of L-929 nuclei that were used for apoptosis analysis. The response of the L-929 cell nuclei to paclitaxel was to swell and fragment. The graphs show the quantitative response of several cellular and molecular parameters to paclitaxel. In each field of cells, nuclear brightness, area, and shape were measured as well as cytoplasmic actin polymerization and mitochondrial potential. The differential response of the normal and transformed cells to paclitaxel is shown. Cross-correlation of multiparameters will yield detailed information in HCS.

## SUMMARY

HCS is a new approach to easing some key bottlenecks in the drug discovery process. Complex information on target distribution and activity in cells is the next frontier in drug discovery. HCS is an important tool for functional genomics in the target validation process. In addition, HCS offers detailed cellular information for lead optimization. New reagents, algorithms, screens, and bioinformatics will increase the power of this approach.

## FUTURE DEVELOPMENTS

### *The CellChip™ system*

Miniaturization and higher throughput tools are two of the three major driving forces that are involved in improving the productivity of the early drug discovery process. It is our perspective that a variety of biological chips will replace the microtiter plate footprint within the next few years. Recently, HTS of nucleic acids and polypeptides has been achieved using high-density arrays of these molecules on chips.[20–22] Typically, DNA or polypeptide molecules are attached in defined locations or spots in an array of up to tens of thousands in number on a small glass or plastic chip. The location of the spot in the array provides an address for reference after the plate has been screened (e.g., the hybridization of fluorescently labeled DNA probes to an array of sequences attached to the chip). The information provided by an array of either nucleic acids or amino acids bound to a substrate is limited according to their underlying "languages." For example, DNA sequences have a language of four nucleic acids and proteins have a language of about 20 amino acids. In contrast, a cell that includes a complex organization of biological materials has a vast "language" with a concomitant multitude of potential interactions with a variety of substances. Thus, a need remains for a high throughput device for HCS of the physiological response of cells to biologically active materials, including candidate drugs.

Previously, arrays of cells have not been produced on chips for screening. However, methods have been described for making micro-patterned arrays of cells for other applications.[23,24] The CellChip System is produced by employing a combination of self assembly monolayer technologies (SAMs) and further molecular modification of the "virtual" wells to yield arrays of cells on glass or plastic (CellChip System). Figure 11 demonstrates an early version of the CellChip technology with one cell type patterned in a 96-well array. The complete CellChip System includes a system of microfluidics to address wells, environmental control to preserve cell function, and a CellChip Reader for optical reading of cell physiology. Furthermore, the entire spectrum of high-content screens developed for the ArrayScan System will migrate seamlessly to the CellChip System. Reducing the well size from the microtiter plate footprint will not only improve the speed and efficiency of scanning for HCS but will also allow for HTS to be performed on the same cell array by first reading the whole area of the CellChip at lower spatial resolution. With this bimodal approach, HTS can

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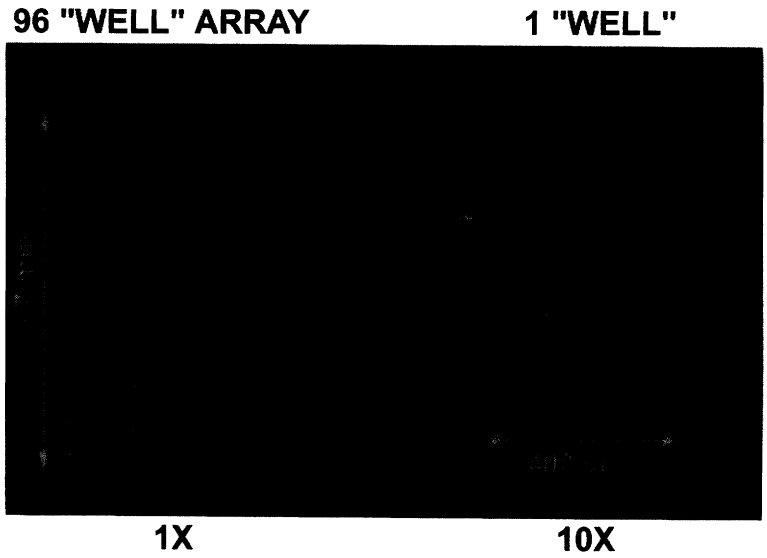


**FIG. 11.** CellChip™ Platform. A 96-well array of cells were patterned onto a glass chip. Freshly trypsinized L-929 fibroblasts were incubated with the CellChip for 15 min at 37°C to allow for attachment of the cells to the engineered wells followed by a wash step. This demonstration chip was labeled with the fluorescent actin label, rhodamine-phalloidin, so that all cells and wells exhibit fluorescence. At low magnification, the whole CellChip can be analyzed in a HTS using one channel of fluorescence. At higher magnification, on the same reader, individual wells can be identified and analyzed for HCS. The CellChip System will increase the throughput of HCS, decrease the volume of reagents and compounds required for screening, and combine HTS and HCS on one platform. Furthermore, CellChips can be engineered to analyze a variety of cells on the same chip.

be directly coupled with HCS on the same platform. In effect, HCS also becomes HTS on the CellChip System.

### *The Cellomics™ Database*

Increased information is the third driving force involved in improving the productivity of the early drug discovery process. Genomics and proteomics have evolved as powerful tools for data management and data mining. The next frontier is the cell with its complex information. The Cellomics Database is a bioinformatics application that involves the systematic development and application of techniques for managing and analyzing data contained in databases built through HCS, modeling, and database search regarding multidimensional cellular information.

To maintain life processes, cells must manage the multidimensional information that interconnects the myriad chemical and molecular activities encased within them. The scale of this living meshwork is daunting. A simplified example demonstrates the scale of the challenge: A conservative approximation is that the average human cell is spherical with a diameter of 10 $\mu$m (~0.5 pl) and it contains 100 mg/ml protein with an average molecular weight of 40,000. These assumptions indicate that each cell contains approximately 1 billion molecules of protein. Because it is believed that proteins mediate nearly all molecular activity in the cell,[25] then there are more than a billion chemical reactions possible just for proteins. Adding temporal and spatial variations, combinatorial reactions of proteins, other classes of macromolecules, and organelles suggests the incredible amount of information inherent in cell functions. This enormous flux of chemical and molecular information is interconnected in time and space through a hierarchy of cellular organization that includes ionic and small molecule (metabolite) signaling, metabolic pathways, macromolecules and their associated structures, and organelles.

The Cellomics database has been designed to capture these complex interrelationships by defining the temporal and spatial dynamics of intracellular molecular processes. The Cellomics database combined the multi-dimensional data from HCS with genomics and proteomics databases into a powerful tool for functional genomics and beyond.

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