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Past, Present, and Future of High Content Screening and the Field of Cellomics

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Summary

High content screening (HCS) was created in 1996 to offer a new platform that could be used to permit relatively high-throughput screening of cells, in which each cell in an array would be analyzed at a subcellular resolution using multicolored, fluorescence-based reagents for both specificity and sensitivity. We developed HCS with the perspective of the history of the development of the automated DNA sequencers that revolutionized the field of genomics. Furthermore, HCS was based on a history of important developments in modern cytology. HCS integrates the instrumentation, application software, reagents, sample preparation, and informatics/bioinformatics required to rapidly flow from producing data, generating information, and ultimately creating new cellular knowledge. The HCS platform is beginning to have an important impact on early drug discovery, basic research in systems cell biology, and is expected to play a role in personalized medicine.

Key Words: Bioinformatics; cellome; cellomics; fluorescence; high content screening; informatics; light microscopy; multiplexed fluorescence; reagents; systems biology; systems cell biology.

1. Introduction

My cofounders and I formed Cellomics, Inc. in 1996 to create a platform technology that would permit large-scale screening of cells, with subcellular spatial resolution, using multiplexed fluorescence in arrays of cells on either microplates or other substrates such as chips ***(1)***. In the years since the introduction of high content screening (HCS), there has been a growing acceptance of the technology in both drug discovery and basic biomedical research markets and numerous companies are now offering various components of a complete platform. This chapter is designed to give insights into how HCS was conceived and implemented in the past, the present state of evolution of the technology, and what the future holds for this rapidly emerging field.

There have been a variety of definitions of terms related to HCS over the last few years since we first introduced the field. The following definitions are based on the early perspectives of the cofounders and early employees of Cellomics and are relevant to today's use of the technology.

1.1. Definitions

Cellome: The complete complement of all cell types in an organism and their constituent molecules.

Cellomics: The study of the dynamic functions of cells and their constituent molecules.

High content screening: Platform and methods, including instruments, biological application software, reagents, assays, and informatics software used to automatically screen and analyze

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arrays of cells to define the temporal and spatial activities and functions of cells, and their constituents, on a cell-by-cell basis, including subcellular features.

HCS assays: The integration of the optimal biological application software with the optimal fluorescence-based reagents and protocols used to extract the type of cellular data defined by a particular experiment on the desired cell type(s).

Multiplexed HCS assays: HCS assays in which multiple parameters are not only measured within single cells using multiple reagents and morphometrics, but relationships between the parameter values are calculated, analyzed, and interpreted on a cell-by-cell basis. It is also possible to make a population average of any or all of the parameters measured on a cell-by-cell basis for some analyses.

Systems cell biology: The understanding of how the integration of the complex biochemical and molecular processes, occurring in time and space, are responsible for cell functions and the complex behavioral responses of cells to natural environmental changes or experimental treatments. The integration can occur within one or more cell types incorporated into an assay and involve panels of multiplexed HCS assays extracting up to hundreds of cellular measurements.

1.2. Why Do We Need HCS?

HCS was developed to meet the needs of research scientists in both basic biomedical research and early drug discovery. In basic biomedical research, the human genome project has identified approx 20,000–25,000 human genes that code for proteins. This coding portion of the human genome represents only about 2–4% of the total genome; the remaining 96–98% was originally assigned "junk" DNA status by many scientists, as a byproduct of evolution *(3,4)*. Over the last few years there has been a drive to define the pathways formed by interactions of the proteins encoded by the coding portion of the genome. These interactions, or the protein "interactome," are believed to bring about specific cell functions. It has become evident that the cell consists of many interacting pathways that are highly regulated. Hundreds of interacting proteins involving a variety of post-translational modifications create a complex network of activity that is only partially defined and understood *(5)*.

As if the complexity of the protein interactions and regulation was not difficult enough, the "new" genomics of noncoding RNA (ncRNA) has recently caused researchers to pursue the apparent parallel world of ncRNA in regulating cell functions. It has been established that about 50% of the human genome is transcribed into RNA, whereas only a small fraction codes for proteins. Recent studies have demonstrated that a growing number of ncRNA exhibit functions like proteins in regulating gene expression and even developmental changes *(6)*. Now it is clear that the proteome and ncRNA species must be investigated together in order to more fully understand cell functions and their regulation. This increases the value and importance of HCS, especially with multiplexed assays to define the functions of proteins and ncRNAs, as well as other cellular constituents, within the context of the living cell system.

Early drug discovery steps traditionally used primarily homogeneous, solution assays containing the protein targets, because they were relatively simple "mix and read" assays. This approach was compatible with the prevailing view in the 1990s that stressed fast measurements on a growing list of targets with large numbers of compounds. The chosen metric was how fast a plate could be read on a plate reader. However, the implementation of ultrahigh throughput screening did not have the desired impact on the number of investigative new drugs generated by this approach. In fact, the productivity of the whole pharmaceutical industry has decreased over the last couple of decades leading industry leaders to make changes in the process of drug discovery *(7)*.

An alternative approach of generating deep, functional information based on screens using cells with temporal and spatial information was suggested, based on HCS *(1)*. Now the metric is how much time is required to make a good decision on whether to continue pursuing a compound.

HCS, especially with multiplexed assays, should play an increasingly important role in drug discovery. In fact, HCS has opened the opportunity to perform drug discovery, not just on a preselected target, but to screen for compounds, singly or combined, that impact cellular functions such as cell cycle, cell motility, apoptosis, and so on *(8,9)*.

1.3. The Cell: First Level of "Systems Biology"

It is a fact that using cells in the early drug discovery process is more complicated and expensive than performing homogeneous protein assays in screens. However, cells offer the first level of the complexity that living systems exhibit and results using cells are more meaningful than those obtained from isolated proteins. In addition, cell-based assays are less complex and expensive than using whole organisms. There is great potential in performing "systems cell biology" screens on the optimal cells (validated cell line or primary cells) and then apply the systems cell biology information as a bridge to higher order systems biology studies. Today, most HCS is performed on two-dimensional (2D) arrays of cell lines. However, more complex cell-based assays can be performed on 2D and 3D cocultures of different primary cell types using tissue-engineering approaches to create functional arrays of tissue models. Information and knowledge gained at this next level of complexity can then be related to higher order systems biology studies.

The amount and quality of information and knowledge that can be obtained by cell-based discovery far outweighs the higher upfront costs in early drug discovery. In addition, the real costs in drug discovery increase as a compound continues down the pipeline. Better, deeper information early should become the new standard. High throughput HCS using multiplexed HCS assays with advanced reagents and informatics will play a major role in this paradigm shift. Success at this level will increase the need and demand for sophisticated systems biology databases that will be populated by mining the literature and the information derived from systems cell biology screens.

1.4. The Concept and Development of the Field of "Cellomics" Mirrored the Developments in the Field of Genomics

The field of genomics was driven by the need to sequence the genomes of organisms in order to understand the complexity and regulation of life processes starting with the DNA "blueprint" of life. Manual DNA sequencing by gel electrophoresis, "reading" the ladder patterns that defined the sequence and then manually entering sequences into spreadsheets was a major development in biotechnology and became a well established method by the late 1970s. Fundamental knowledge about selected genes and genome organization was created by the manual processes involved in this early approach to DNA sequencing. However, the human genome project demanded that automated instrumentation, with the optimal reagents and informatics/bioinformatics software tools be developed to permit the human genome to be defined in a reasonable period of time and cost. In the early 1990s, Applied Biosystems (Foster City, CA), as well as others, developed "complete" solutions to automatically prepare the DNA samples, fluorescently tag the four nucleotides, run the gels, read the ladders, and then read-out the sequence into searchable databases. Bioinformatics tools rapidly evolved to identify genes in the growing genome sequences *(10,11)*.

The field of cellomics was driven by the need to define the functions of genes and the proteins that they encoded. It was apparent by the mid-1990s that knowing the human genome was the start, not the end of the biological challenge for basic research and drug discovery. Light microscopy, especially digital imaging fluorescence microscopy on living cells was chosen as the best approach to defining the functions of genes and proteins *(1)*. Human interactive, imaging methods were pretty well developed by the 1980s and fundamental information about the temporal and spatial dynamics of cells and their constituents was being published by a growing academic community *(12–18)*. However, the human interactive imaging tools in the absence of automated imaging methods and informatics tools to archive, mine, and display complex

imaging data made the process of studying cells time-consuming and complicated. Similar to the field of genomics, there was a need for the development of an automated system to acquire, process, analyze, display, and mine massive amounts of cellular data derived from arrays of cells treated in various ways. In 1997, Cellomics, Inc. offered a "complete solution" with the introduction of the ArrayScan platform that consists of the instrument, biological application software, reagents for a specific assay, and the first generation informatics for cell analyses. This was the starting point for the large-scale investigation of the function of genes and the proteins they encoded, as well as other cellular constituents *(1)*.

2. Past: Origins of HCS

2.1. Important Milestones in the Modern Era of Cytology and Cytometry

The development of HCS is rooted in the rich history of developments in cytometry going back more than 50 yr in the "modern" era of cytology. **Figure 1** depicts this authors view of the major advances that occurred over the last 50 yr that led up to the development of HCS. There have been many important developments over this period of time and not all of the important ones are depicted here. The development of immunofluorescence microscopy by Coons and Kaplan *(19)* was the first critical step in the modern era of fluorescence-based cytometry. For the first time, the specificity of labeling with antibodies was coupled to the sensitivity of fluorescence detection. Interestingly, a major advance in cytometry occurred in 1957 with the discovery of confocal scanning microscopy by Minsky more than 30 yr before optimal fluorescence dyes and imaging technologies made the method practical for fluorescence microscopy *(20)*. The early stages of the modern era of cytology also includes the development of the inverted fluorescence microscope *(21)* and dichroic filters for epifluorescence microscopy *(22)*, both of which created a system that produced the light throughput and signal/noise required for the practical use of fluorescence microscopy as a standard tool *(14)*.

The next phase in the modern era of cytometry consists of the development of fluorescence-based flow cytometry *(23–26)*, a method that blossomed with the development of specific antibodies to a range of cell surface molecules important in the immune responses and the use of multicolored fluorescent dyes to multiplex the measurements. Also in the late 1960s was the development of image intensifiers, imaging detectors that could record images of very low fluorescence signals in biological samples *(27,28)*.

The late 1960s and into the 1970s was a period of rapid developments in instrumentation *(14,29,30)* and imaging software *(31–33)*. Fluorescence-based reagents also emerged as a critical component of the detection systems (**Fig. 2**). In particular, Alan Waggoner created the modern field of fluorescence-based physiological indicators with the development of a series of membrane potential sensitive dyes *(34)* and Haugland *(35)* developed and/or commercialized a wide range of physiological indicator dyes. In addition, fluorescent analog cytochemistry, the original tool to measure the activities of specifically labeled proteins in living cells in time and space, was demonstrated *(36,37)*. However, the production of fluorescent analogs was a time-consuming process including protein purification, labeling, testing function in vitro and microinjecting into living cells *(37,38)*. Wide-spread use of this technology would require another technical development in the 1990s.

The 1980s were characterized by major developments in video microscopy to enhance the contrast and detection limits in both transmitted light and fluorescence *(12–15,39)*, and ratio imaging microscopy to quantify cellular physiological changes *(40,41)*. Ratio imaging was initially applied to pH *(40,41)* and then free calcium ion concentration *(42)*, but ultimately local protein concentrations and activation *(40,43)*, as well as cytoplasmic structure and rotational diffusion of proteins *(44)* (**Fig. 3**). The use of solid-state detectors improved the performance of imaging methods *(45)*, and the first practical use of laser scanning confocal fluorescence microscopy allowed 3D imaging of thicker biological samples *(46)*.

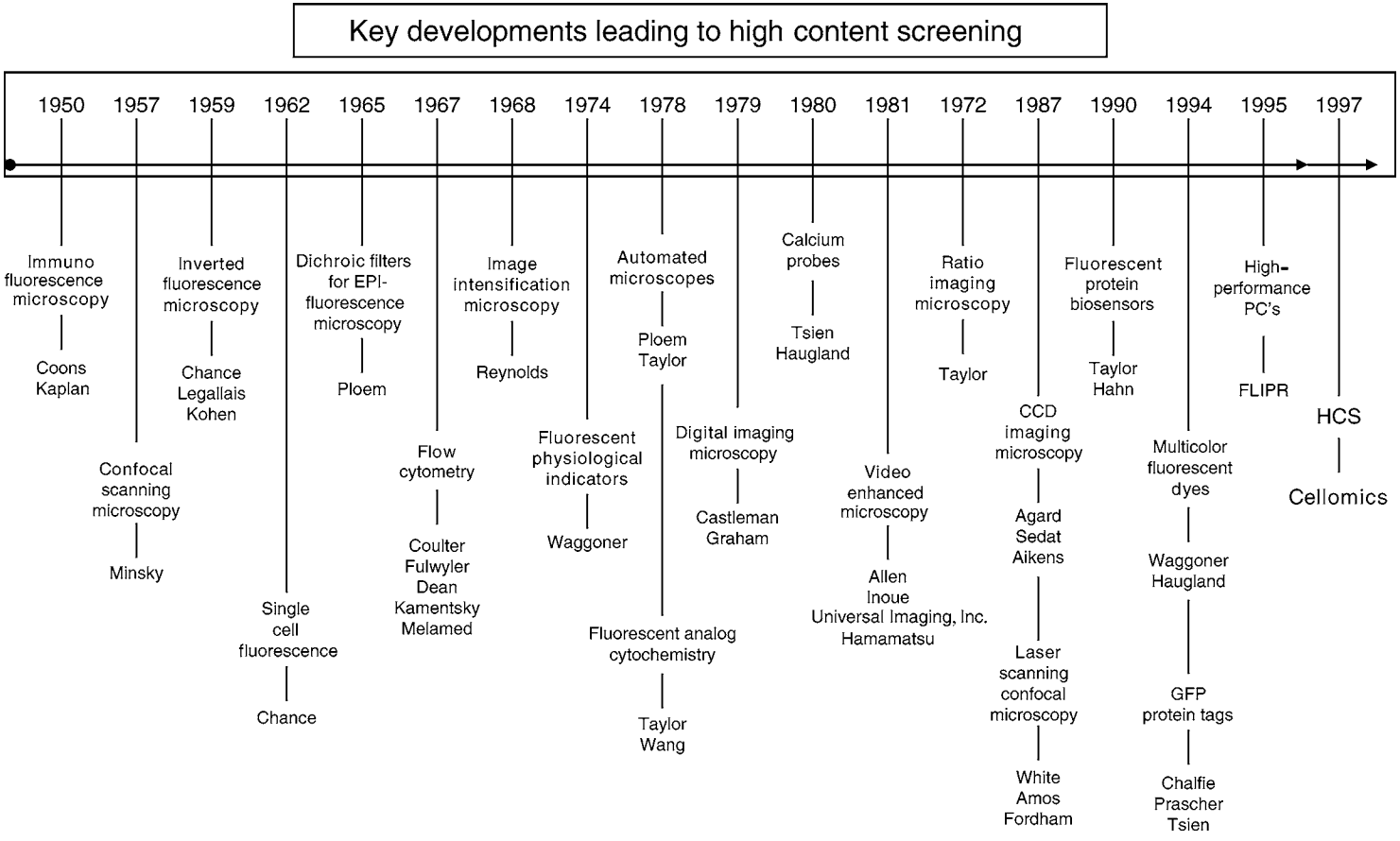

Fig. 1. Time line of the key developments that have occurred since 1950, the beginning of the modern era of cytometry. High content screening was based on a variety of important contributions that led to the development of HCS in 1997.

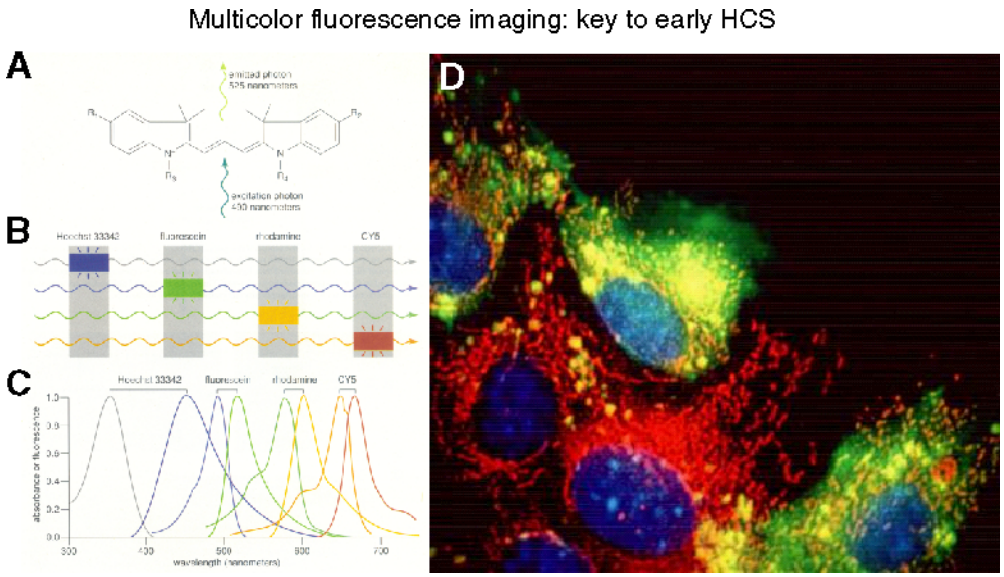

Fig. 2. Multicolor fluorescence imaging has been a key to the early stages of HCS. **(A)** Fluorescent molecules have a common motif in their structure that includes a pattern of alternating single and double bonds between carbon atoms, especially in ring structures. This example is a cyanine dye. **(B)** Multiple fluorescent dyes can be used in cells and can be distinguished with the use of optimal filter sets to produce multiparameter data sets or multiplexed measurements. **(C)** The goal is to assemble multiple fluorescent dyes in which the spectral overlaps are minimized so that filter sets can separate the distinct dyes. Recently, spectral deconvolution systems have been developed to extract the fluorescent spectrum of each dye rather that to separate them with filters. **(D)** An image of living mouse fibroblasts that have been labeled with a dye to label the DNA in nuclei (blue), a molecule that was endocytosed into endosomes (yellow), a dye to label and report the membrane potential of mitochondria (red) and labeled actin incorporated into the cells (green).

The early 1990s ushered in the era of advanced fluorescence-based reagents. Multiplexed imaging with water soluble, bright, stable, fluorescent dyes was optimized by the development of a range of cyanine dyes that emitted fluorescence from the blue to the near infrared portion of the light spectrum *(47)*. It was now possible to correlate multiple cellular parameters in the same cells *(48)*. The development of fluorescent analogs of proteins in the late 1970s *(36)*, led to the creation of fluorescent protein biosensors, reagents that reported biochemical or molecular changes, not just their distribution within the cell *(49–53)*. The original green fluorescent protein gene construct from the jelly fish (*Aequoria*), was also first used in a biological experiment *(54)*. Tsien et al. *(55)* subsequently optimized the properties through the selection of mutants making this the method of choice for creating fluorescent analogs of proteins.

My cofounders, early employees, and I were primarily influenced by three major types of fluorescence instrumentation used in cellular analyses in the creation of HCS (**Fig. 4**). First, flow cytometry created a platform in which multiplexed measurements of cells could be performed. Flow cytometry opened the door to fluorescence-based analyses and became a standard for cell-by-cell measurements in basic research and biotechnology *(23)*. Second, the fluorescence imaging plate reader (FLIPR; Molecular Devices, Inc., Sunnyvale, CA) the first whole plate reader designed for high throughput measurement of population averages of attached living cells, offered cell analyses as a powerful approach to drug discovery *(56)*. Finally, digital imaging microscopy created a tool that allowed multicolored and even multimodal microscopy to be used by biologists, not just biophysicists *(17)*.

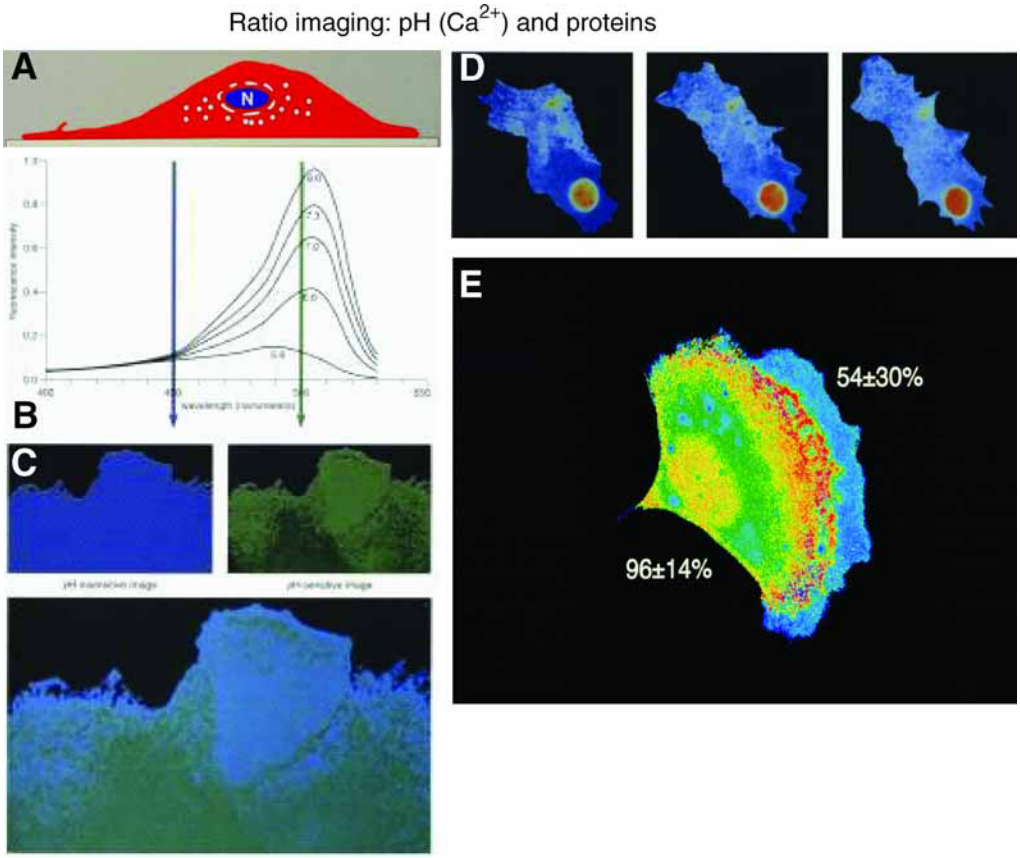

Fig. 3. Ratio imaging is a powerful quantitative method to measure ion concentrations and relative protein concentrations, as well as other parameters (*see* **Table 2 [A]**). Diagram showing the cross-section of an average mammalian cell. The variable pathlengths at different points in the cells, as well as the "accessible volume" of cellular structures that exclude labeled molecules require a ratiometric method to normalize the measurements. **(B)** Fluorescence excitation of the pH sensitive dye, BCECF at 500 nm shows a strong pH sensitivity, whereas excitation at 450 nm shows no pH sensitivity. **(C)** Image pair when a row of cells loaded with BCECF is excited at 450 and 500 nm and then the resultant ratio image that shows a small gradient of pH. **(D)** A series of ratio images, taken over a few seconds, of a cell that had been loaded with a calcium sensitive dye. The nucleus maintained a higher free calcium ion concentration (red color), whereas the cytoplasm exhibited patterns of elevated free calcium as new cell extensions formed. **(E)** A ratio image of a living mouse fibroblast that was coloaded with a labeled myosin II motor protein and a distinctly labeled dextran used as a volume marker. The leading edge of migrating cells excluded myosin II as demonstrated by the low ratio value (right side of cell) that is depicted by a blue pseudocolor. Myosin II is concentrated behind the leading edge of migrating cells as shown by the high ratio value depicted by the red pseudocolor. The myosin II exhibits a reduced diffusion in which the myosin is at a higher concentration relative to a soluble dextran as measured by fluorescence recovery after photobleaching (54 ± 30% of the myosin recovers). The myosin II exhibits almost 100% recovery in the posterior of migrating cells. Myosin II appears to assemble just behind the leading edge, translocate toward the posterior of the cell, contract and then the myosin II disassembles for the continuation of this cycle, as the cell migrates ***(64)***.

Although the human interaction required to operate the digital imaging microscope systems was a limitation, these early systems were responsible for generating some very important insights into the dynamics of living cells ***(12–18)***. However, the experimental time domain for progressing from producing data to generating information and then creating new knowledge from relatively small

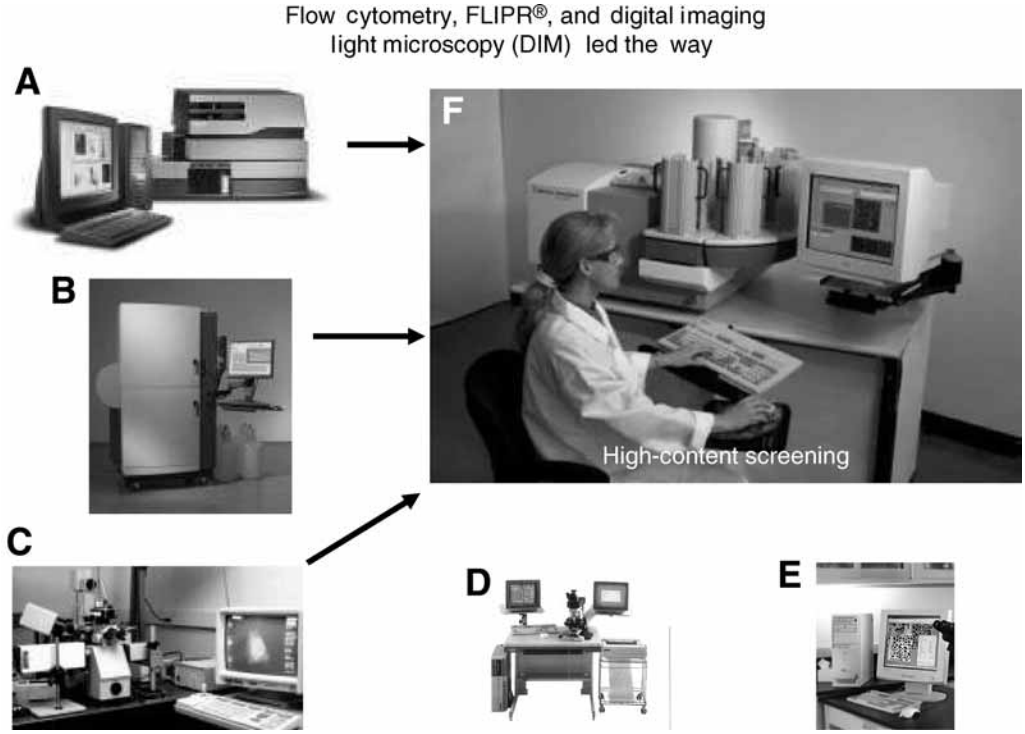

Fig. 4. Flow cytometry, FLIPR and digital imaging light microscopy led the way to the development of HCS. **(A)** Flow cytometer for cells in suspension (e.g., Beckton Dickinson, Mt. View, CA). **(B)** FLIPR instrument to measure the population average fluorescence of cells within wells of a microplate (Molecular Devices, Sunnyvale, CA). **(C)** Multimode light microscope commercialized by Biological Detection Systems, Inc. to study the temporal and spatial dynamics of living cells *(17)*. **(D** and **E)** show other early imaging systems from Cell Analysis Systems, Inc. and Universal Imaging, Inc. **(F)** The first generation ArrayScan HCS instrument developed by Cellomics, Inc., Pittsburgh, PA.

Table 1
Major Modes of Digital Imaging Light Microscopy *(14,15)*

Mode of microscopy	Information gained
Video-enhanced, differential interference contrast	2D and 3D dynamics of cells, organelles and structures with subresolution detection
Polarized light	Molecular anisotropy without labels in cells with nm detection
Fluorescence	2D and 3D dynamics of molecules, organelles, and cells with molecular specificity, up to single molecule sensitivity and spectroscopic measurements

cell samples was from weeks to months. The early commercial entries included the Multi-Mode Imaging Microscope offered by Biological Detection Systems, Inc. *(17)*, the MetaMorph imaging software offered by Universal Imaging, Inc. (now part of Molecular Devices, Inc.), as well as others. **Table 1** summarizes the major modes of digital imaging light microscopy and **Table 2** summarizes the distinct modes of fluorescence microscopy available by the mid-1990s.

Table 2
Fluorescence is the Most Powerful Mode of Light Microscopy *(14–18)*

Mode of fluorescence	Information gained
Full-field and confocal–multicolor fluorescence	Spatial-temporal correlation of the distribution of cell constituents
Fluorescence speckle microscopy	Motion tracking of structures and assembly-disassembly of structures
Ratio imaging microscopy	Quantitative measurements of ion and relative protein conc., energy transfer, and steady-state fluorescence anisotropy
Fluorescence recovery after photobleaching and photo-activation of fluorescence	Quantitation of molecular transport and diffusion
Microtomography	3D and 4D distribution of labeled structures
Total internal reflection fluorescence	Molecular dynamics constrained to the interface of cells and a substrate
Fluorescence anisotropy imaging	Molecular binding and rotational diffusion
Multiphoton laser scanning confocal	Imaging deep into thick samples
Standing wave fluorescence	Spatial resolution down to 30–50 nm in thin samples
Fluorescence lifetime imaging	Direct measurement of lifetime of the excited state to measure molecular binding, rotational diffusion and energy transfer, independent of fluorescence intensity

3. Present: HCS Meets the Challenge by Automating Cell Biology

3.1. A Fundamental Change in the Microscopic Analyses of Cells

HCS made a major step beyond the prevailing methods of digital imaging light microscopy similar to the advance of automated DNA sequencing over manual sequencing methods. This was accomplished by automating the major aspects of the imaging process, including the analyses of huge numbers of arrayed cells that could be tested with a wide range of experimental treatments rapidly and without extensive human interaction. Automation of image acquisition, image processing, image analysis, image archiving, and image visualization made it possible to prepare large numbers of microplates, place them in a stacker on the HCS instrument and then walk away while the plates were processed by the system. This permitted an accelerated approach to the process of producing data through creating new knowledge from a massive number of cells in a matter of 1 d. This fundamentally changed the process of doing large-scale cell biology in basic biomedical research and drug discovery.

The small-scale imaging-based cell analysis that was permitted by the early digital imaging light microscope systems was labor intensive, used a relatively small number of cells, and also focused on creating images as the most important data output. In contrast, the large-scale imaging-based cell analysis permitted by HCS was fully automated, could be easily applied to large numbers of cells with parallel sample analyses, and focused on automatically converting the image data into digital data and generating cellular information that would lead to creating new cellular knowledge (**Fig. 5**). It is important to note that a continuum of approaches from using small-scale imaging to HCS is often required to fully investigate a biological problem.

3.2. The Major Elements of HCS

It was apparent to us at the beginning of the development of HCS that the whole process was a systems engineering challenge. The major components of HCS are depicted in **Fig. 6.** The complexity of moving through data production, information generation, and knowledge creation

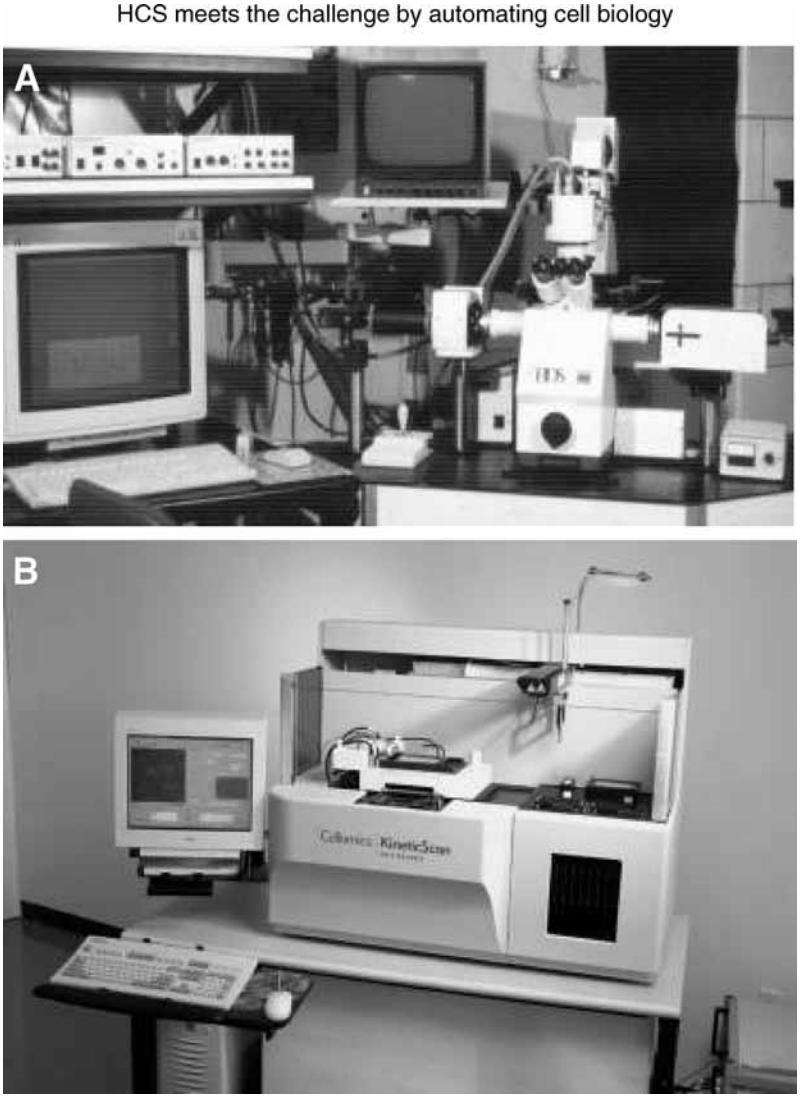

Fig. 5. HCS meets the challenge to create a high throughput platform to automate cell biology. **(A)** User-interactive, substantially manual, digital imaging microscopy was a precursor to high content screening. These imaging systems were labor intensive, because the operator had to continually interact with the imaging system. The sample sizes were usually small in which anywhere between a few and a few hundred cells were studied within a day of experimentation. The focus on these early imaging systems was image data, including time-lapse "movie" sequences. **(B)** HCS introduced fully automated imaging of large numbers of cells treated in a combinatorial fashion. The automation permitted large numbers of cells to be investigated within 1 d (ranging from approx 10^4 to 10^7/d). The new focus has been away from simply producing images to automatically generating information and creating new knowledge from the image data sets.

required the development and implementation of a complete solution *(57)*. The whole process of HCS starts with the biological question that can be addressed with the development of an optimal assay, which is the integration of the right cells, reagents and application software. Presently, most HCS measurements are preformed with directed algorithms based on preknowledge of the biological domain information. However, undirected algorithms or pattern recognition software

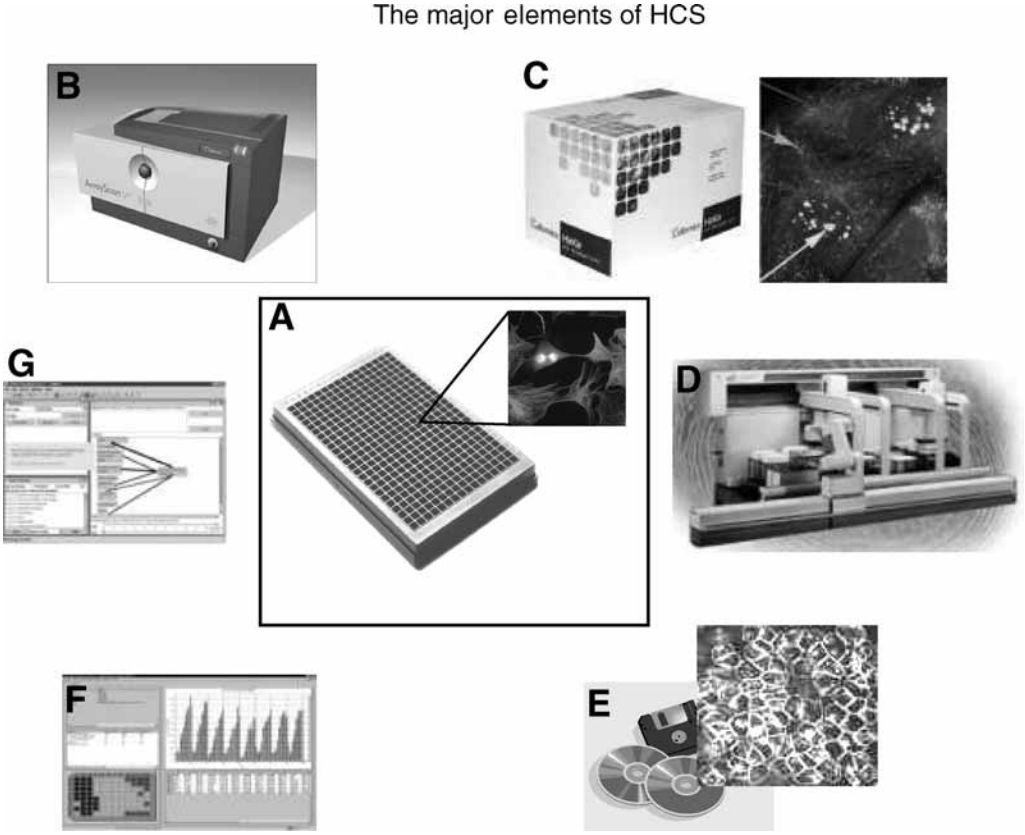

Fig. 6. HCS involves **(A)** the creation of arrays of cells, **(B)** instrumentation, **(C)** reagents, **(D)** sample preparation, **(E)** biological application software, **(F)** informatics software, and **(G)** cellular bioinformatics.

can be used, especially when the optimal cell parameters required for understanding the images are not specifically known. Both types of application software tools will continue to evolve (*see* Chapters 5 and 6).

HCS deals with large numbers of cells that are arrayed for combinatorial treatments, presently in microplates. In order to process large numbers of microplates and experimental treatments, it was critical to implement automated sample preparation for speed and reproducibility. The HCS instrumentation allows the reading of whole microplates in minutes, depending on the assay type, usually with the application software running on the instrument. However, it is currently possible to acquire images and then apply selected algorithms offline ***(57)***.

Even a small screen can create hundreds of gigabytes to terabytes of data within a short period of time. Therefore, data storage (images, metadata, and numerical data), coupled with a variety of informatics software tools, were required to actually perform significant screens. Although presently limited in scope, cellular bioinformatics tools have also begun to evolve in order to help create knowledge from the information gleaned from the data using the application software and informatics software tools ***(2,57–59)*** (*see* Chapters 22–24).

3.3. Fixed End Point vs Live Cell HCS

All HCS assays begin with living cells that are treated with some combinatorial of manipulations including small molecules, biologicals, and/or physical treatments. Cells and plates that are fixed at some time-point after experimental treatment and then subsequently washed, labeled and

read on an HCS instrument are called "fixed end-point assays." The sample preparation methods can automate all of these steps making it very fast and reproducible. However, the time domain of the biology is limited to a single time-point. Therefore, the investigator must either create a whole time-course by preparing multiple plates processed over time or initially define the half time of some cellular process of interest and set the time of fixation accordingly. The fixed end point approach can be a relatively high-throughput screening method *(57)* (*see* Chapters 2, 8, 13, and 26–28).

Live cell HCS is possible with the incorporation of on-board fluidics and an environmental chamber into the HCS reader. This can be accomplished with either a distinct instrument or by applying add-ons to a fixed end point reader *(2,57)*. Live cell assays can also be based on a single time-point with the use of fluorescent probes that are functional in living cells, or full kinetic measurements can be made, over time, starting before the experimental treatment and continuing over a defined period of time *(60)*. Kinetic assays are critically important in order to define the half-times of specific biological processes before setting up fixed end-point assays and/or for critically defining the complex temporal/spatial dynamics of cells and their processes.

4. Future Directions

HCS is still in its infancy. All of the elements of HCS depicted in **Fig. 6.** will evolve over time. Assays will become heavily multiplexed in which many cellular parameters will be measured in parallel in order to create a "systems cell biology" profile of cellular functions. The application software will be more powerful and will include elements of both directed algorithms and machine learning. A major direction will include the development of many classes of reagents that will "measure and manipulate" cellular constituents from DNA through all types of coding and ncRNA, proteins and metabolites. It will be possible to manipulate pathways and to measure the impact of the presence or absence of specific molecules on cell functions. A real systems cell biology profiling capability will emerge (*see also* Chapters 11, 12, 14, and 16–19).

The types of arrayed cells, the substrate structure, as well as the biology and chemistry of the environments will become more physiologically relevant. For example, biologically significant substrates using extracellular biochemistry will replace simple cells on plastic that now dominates the field. In addition, primary cells will be used to a greater extent based on improved methods for preparing and transporting these cells (*see* Chapter 9). Rather than investigating one cell type in 2D per well, the future will yield "tissue engineered" arrays of cells that have some tissue functions based on the optimal arrangement of specific types of cells (*see* Chapter 10). Further miniaturization will occur and cell chips will be engineered using a combination of nanotechnology and microfluidics *(1)*. Finally, it is not a distant vision to predict that cells will come stabilized (by freezing or freeze drying), prepackaged and containing a variety of biosensors ready for activation and screening.

The instrumentation will become more sophisticated. The next generation of instruments will be modular, allowing the end-user to define the specifications required for the desired applications. Options will include distinct types of light microscopy (**Table 1**) and multiple types of fluorescence measurements beyond intensity (**Table 2**). It is predicted that because the format for HCS will be miniaturized to chips, the instruments will be smaller and faster based on this transition. Finally, future generations of instruments will incorporate standards including intensity, spectral correction and size. These instrumentation standards will also be linked to standards incorporated into the cell array formats (*see* Chapters 4 and 7).

The informatics tools will become more sophisticated and automated to handle the massive cell data streams (*see* Chapter 20). These tools will include powerful data mining tools to extract patterns from multiplexed assay data sets, cell pathway tools to map the interactions of

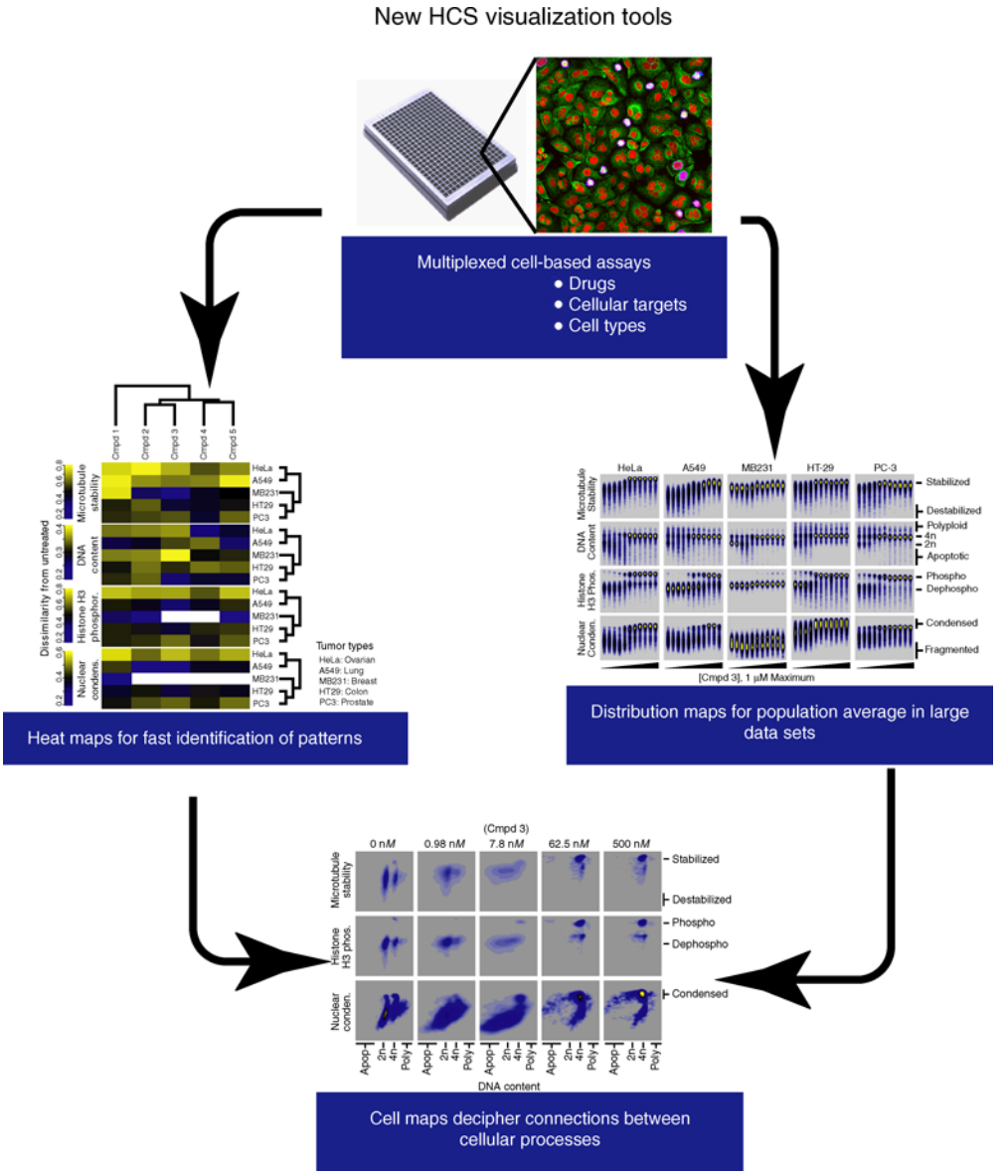

Fig. 7. Multiplexed HCS assays allow complex cell functions to be analyzed rapidly and in great detail. A complete systems cell biology knowledge building platform will include a continuum of software tools starting from the imaging algorithms to data archiving, mining, analysis, and cellular bioinformatics tools to rapidly traverse from collection of data, through the generation of information and the creation of knowledge *(2)*.

cell constituents, as well as visualization tools to rapidly detect important information for further exploration (**Fig. 7**) (*see* Chapters 22 and 24).

The entire work flow will become more integrated and continuous from growing cells, plating cells, incorporating reagents into cells, followed by acquiring the data with the instrumentation and application software, to the archiving, analysis, and visualization of data and information. In addition, the final step of interpretation of the information to create new

knowledge will use cellular bioinformatics software. This integration will require more advanced software tools including advanced informatics and bioinformatics.

The field that was started 10 yr ago is only now beginning to blossom. It is my opinion that the greatest challenges and opportunities in HCS will involve the development and application of advanced reagents and informatics/bioinformatics tools. The next 10 yr will usher in tremendous opportunities for HCS-based discovery in basic biomedical research and drug discovery, but will also impact some industrial testing and personalized medicine. It is expected that in vitro toxicology will be the next major area of development that could ultimately produce a predictive tool ***(61–64)*** (*see* Chapter 30).

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