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A Personal Perspective on High-Content Screening (HCS): From the Beginning

D. LANSING TAYLOR

High-content screening (HCS) was introduced in 1997 based on light microscope imaging technologies to address the need for an automated platform that could analyze large numbers of individual cells with subcellular resolution using standard microplates. Molecular specificity based on fluorescence was a central element of the platform taking advantage of the growing list of reagent classes and the ability to multiplex. In addition, image analysis coupled to data management, data mining, and data visualization created a tool that focused on biological information and knowledge to begin exploring the functions of genes identified in the genomics revolution. This overview looks at the development of HCS, the evolution of the technologies, and the market up to the present day. In addition, the options for adopting uniform definitions is suggested along with a perspective on what advances are needed to continue building the value of HCS in biomedical research, drug discovery, and development and diagnostics. (*Journal of Biomolecular Screening* 2010:720-725)

Key words: high-content screening, fluorescence, cellular systems biology, informatics, imaging, cell-based assays, digital imaging, data analysis software

INTRODUCTION

It is fitting that the *Journal of Biomolecular Screening* (*JBS*) has produced a special issue on high-content imaging, screening, and analysis. The original paper on high-content screening was published in *JBS* in 1997[1] by an invitation from one of the editors of the journal, Carol Ann Homon from Boehringer Ingelheim, to submit an article on high-content screening. At the time, I was a cofounder and CEO of a small biotechnology company called BioDx that changed its name to Cellomics in 1998 as part of a financing event. In late 1996, I was beginning to launch BioDx/Cellomics and started to raise capital while taking a leave of absence from Carnegie Mellon University, after years of basic biomedical research involving advanced fluorescence microscopy, imaging technologies, and reagents for live-cell dynamics. While at Carnegie Mellon, we had a valuable collaboration with Merck, and Dutch Boltz was the key contact from Merck looking at how to measure the activation of NF-κB in cells. Dutch is presently doing research on infectious diseases at the US Army Medical Research Institute of Infectious Diseases. It became clear that the pharmaceutical industry would benefit from a platform that could yield better, functional information in the discovery process with an automated, cell-based imaging platform. Although my academic colleagues and I were in a research center focused on this type of technology, the development of a whole, robust platform would require a focused commercial effort. We had some imaging software remaining after the sale of our previous company, Biological Detection Systems, Inc., as a starting point, so we decided to launch. The human genome project was in full tilt, and doing the same kind of analyses on cells was possible, but industrial-scale tools were required.

Cellomics developed a platform based on light microscope imaging technologies to address the need for an automated platform that could analyze large numbers of individual cells with subcellular resolution using standard microplates. Molecular specificity based on fluorescence was a central element of the platform taking advantage of the growing list of reagent classes and the ability to multiplex. A significant patent portfolio was developed during the development phase of the company that was later licensed to other instrument companies to help build the market. We named the approach high-content screening (HCS) to differentiate it from standard high-throughput screening (HTS) platforms. The focus was on developing deep, functional information on cells and subpopulations of cells while scanning plates as fast as possible.

In this timeframe from 1996 to 1997, cell-based assays represented only ca. 10% of the assays performed in the pharmaceutical industry. The focus was on HTS and then ultra-high-throughput screening (UHTS) usually performed as homogeneous assays on isolated targets or cell fractions. In 1996, two talented

Cellumen, Inc., Pittsburgh, PA, USA.

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engineers, Kirk Schroeder and Brad Neagle from NovelTech Systems, Inc., created the Fluorescent Imaging Plate Reader (FLIPR) to solve a problem outlined to them by Vince Groppi, then at Upjohn, to measure a population average response of living cells plated in a 96-well plate. The initial assay used cells labeled with a fluorescent membrane potential sensitive probe. The paper introducing the FLIPR was published in *JBS* in 1996,[2] and it was clear that this platform was complementary to our HCS platform and would help to drive the use of cell-based assays.

We predicted that cell-based assays would grow dramatically over the next decade, and I believed that the FLIPR platform would be accepted before HCS because it was more like a standard plate reader used in the industry. The FLIPR analyzed a cell population average for each well of a microplate. Interestingly, I contacted Kirk Schroeder and tried to acquire NovelTech Systems as a complementary platform in our plan to build an "industrial-scale" cell-based profiling, platform business. I was about a month late because Kirk had made a commitment to sell his company to Molecular Devices. Therefore, we launched headlong into building the HCS platform that we thought would ultimately have a larger impact but take a bit longer to gain commercial traction. Several generations of the FLIPR and competitive platforms have evolved in parallel with HCS, yielding valuable information on population responses from cells.

This article is not intended to be an inclusive review of HCS because very good reviews and books have been published during the past few years. I do identify some key developments from my personal vantage point and point to some important publications. I was also asked by the guest editors to discuss some of the positive and negative surprises for me over the past 13 years in the life of HCS, as well as my view of the future.

IMPORTANT DEFINITIONS RELATED TO HCS: A COMMON LANGUAGE

Different definitions have emerged over the years to address the evolution of this field. Some of the definitions have been published elsewhere, and various interpretations have been made by the growing list of talented researchers and practitioners in the field. There are no correct or incorrect definitions at this stage, but the field should zero in on some basic definitions as part of the maturation of the field. The following suggestions have some historical and practical basis.[3] In my opinion, the Society of Biomolecular Sciences (SBS) should play an important role for standards, including definitions. It would be particularly valuable to do this as a joint exercise with the International Society for Analytical Cytology (ISAC).

High-content screening (HCS). —platform and methods, including instruments, application software, assays (with fluorescence-based reagents), and informatics software, used to automatically screen and to analyze arrays of cells to define the temporal and spatial activities and functions of cells and their constituents.

Automated microscopy. —was used to define HCS by many investigators, especially academic researchers who migrated from using semiautomated research imaging microscopy systems from the 1980s and 1990s to automated systems defined by HCS. The roots of HCS in earlier basic research microscopy and imaging systems has been well characterized, as well as the correlation between automated DNA sequencing and HCS versus manual DNA sequencing and semiautomated imaging systems.[3]

High-content analysis (HCA) and high-content imaging (HCI). —these terms arose around 2002 to address the analysis of any cell image data, whether taken by a research imaging system or an HCS platform, as well as other methods that did not use fluorescence. These terms probably arose due in part to the resistance to the use of "screening" in more academic applications.

HCS assays. —the integration of the optimal biological application software, including unsupervised algorithms and directed algorithms, 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. —the earliest HCS assays were very simple target-centric assays using a DNA label for focusing and cell count and a single target-specific reagent. Multiplexing of a few parameters was a natural progression based on the ability to mix multicolor and/or lifetime fluorescent probes. In HCS assays, multiple cellular parameters are not only measured within single cells using multiple reagents and morphometrics, but relationships between the parameter values are also 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.

Cellome. —the complete complement of all cell types in an organism and their constituent molecules.

Cellomics or cytomics. —the original name of "small c" cellomics was coined to complement the related "omics" fields that preceded cellomics—namely, genomics and proteomics: the study of the dynamic functions of cells and their constituent molecules. Alternatively, cytomics has been used by a variety of vendors and researchers probably due to the overlap of the name of the new field and the company, Cellomics, Inc. One of the two should become a standard.

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Cellular systems biology (CSB). —the investigation of cells as integrated and interacting networks of genes, proteins, and metabolic processes that give rise to either normal or abnormal conditions. CSB involves highly multiplexed panels of functional biomarkers coupled to reference databases and classifier software to convert data into actionable indices.

Label-free analyses. —label-free methods applied to cells arose during the early years of the implementation of HCS and have included the use of transmitted light microscopy, combined with HCS or alone; measurements of electrical impedance; and other optical contrast methods: the measurement of cellular responses without the use of reagents. These methods are complementary to HCS and can be used in specific applications without HCS when the measurements are calibrated with more specific molecular measurements.

EVOLUTION AND PROGRESS IN HCS

The initial paper on HCS published in *JBS* introduced a number of important elements that have been built upon over the intervening years.[1] The key introduction was the first HCS platform, the ArrayScan® that converted semiautomated light microscopy[3,4] into an automated platform that did not require user viewing or intervention once a microplate scan was initiated. The primary output was not images but extracted data on cell functions and parameters. Both live-cell and fixed endpoint applications were demonstrated along with a summary of the classes of fluorescence-based reagents available at the time, including the translocation of a green fluorescent protein (GFP)–tagged glucocorticoid receptor from the cytoplasm into the nucleus. The concept of addressing both target-centric and phenotypic screening was also introduced. The major challenge of managing huge data sets for viewing and analyses was anticipated with the first-generation data management software. The initial miniaturization strategy was also demonstrated with a prototype CellChip along with the concept of the "Cellomics Database" to capture the complex interrelationships between cellular constituents measured by HCS and/or other methods.

The HCS platform was based on many years of basic biomedical research, including the development of research imaging microscopy, imaging technology, and reagents. The history behind the developments has been published.[1,3-7] The early efforts in HCS were focused on target-centric applications, especially fixed endpoint assays, because this was the requirement of the pharmaceutical industry at the time. The two earliest publications on the application of HCS to drug discovery were performed by Cellomics in critical collaborations with Merck on the translocation of NF-κB induced by interleukin-1 and tumor necrosis factor-α[8] and with J&J on the internalization of a GFP-tagged G-protein-coupled receptor.[9]

The first few years of the implementation of HCS put pressure on the growing number of vendors with HCS instruments to improve instrument performance, including image quality, scanning speed, and the addition of new types of assays/screens.[10,11] Multiple vendors launched their own versions of HCS with a variety of differentiation metrics, including optical sectioning and scanning speed. Large instrumentation companies acquired some of the smaller companies that developed the HCS instruments and software such as Praelux/Imaging Research/Amersham/GE, Evotec Technologies/PerkinElmer, Atto-Biosciences/BD, Universal Imaging, plus Axon Biosciences/Molecular Devices (now Danaher). The competitive environment by 2003 was valuable for the end users because competition induced improvements in the HCS platforms and pricing. Cellomics, Inc. agreed to be purchased by Fisher Scientific/Thermo Fisher to remain competitive with much larger companies.

An update on the advances in HCS was also published in 2003.[12] The platforms had evolved to offer a more integrated solution with improved, more user-friendly and flexible software. The ability to add compounds before and during measurements was incorporated into live-cell, kinetic instruments, including the KineticScan Reader from Cellomics, as well as the Attovision from Atto Biosciences. Furthermore, better data management systems were available, and there was the introduction of bioinformatics tools to glean information from the literature. By 2003, academic investigators were actively involved in using HCS to perform large-scale cell biology.[13-18]

Systems biology was emerging as an important field in life sciences early in 2000 and was defined by a number of investigators, including Lee Hood at the Systems Biology Institute (ISB).[19] Lee's definition of systems biology is "the science of discovering, modeling, understanding and ultimately engineering at the molecular level the dynamic relationships between the biological molecules that define living organisms."[19] Systems biology became an important field based on the integration of "omics" information and knowledge to begin exploration of whole organisms.

Several groups identified the potential to treat cells, the fundamental units of life, as the simplest system and to use HCS platforms to generate complex, functional data. This required not only the generation of very large-scale data sets but also the development of analytical and visualization tools to extract information, as well as to manage and to simplify the information so that it could be developed into new knowledge. The application of unsupervised, as well as supervised, image acquisition approaches demonstrated the ability to extract important data from large samples either without prior definition of specific cellular features or by predefining the cellular features.[16,20,21] The application of heat maps to complex cellular data sets, as with previous applications to genomics, allowed a rapid mechanism for looking at patterns in the data that could be explored in more depth; Kolmogorov-Smirnov (KS) statistics could be used to identify population changes compared to a reference state; and other visualization tools could facilitate the

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drilling down from population average data to single-cell correlations of specific features.[15,22,23]

By 2006, there were a large number of both industrial and academic users of HCS. Valuable books containing key reviews on the major elements of HCS and example applications from drug discovery to large-scale siRNA profiling in academia and industry have been published.[24-27] In addition, there have been special issues of journals,[28] as well as reviews, on both general and specific topics that have been published.[29-34] There are now many sophisticated users of existing technologies, as well as developers of HCS-related technologies, that extend the power with advanced instruments, imaging software, reagents, assay protocols, informatics, bioinformatics, and in silico models. The application of HCS has blossomed.

A Cellular Systems Biology (CSB™) approach to drug discovery, drug development, and clinical samples for trials and diagnostics was pursued with the formation of Cellumen, Inc.[35-38] The concept is to use HCS readers to capture data from panels of fluorescence-based, functional biomarkers—usually 8 to 12 molecular parameters selected from information derived from genomics, proteomics, and metabolomics and/or chosen by the use of unsupervised imaging methods to select optimal combinations of biomarkers from dozens of individual biomarkers, the use of optimal types of cells or tissues, reference databases, and the use of classifier software to predict outcomes and to define mechanisms of action. A great deal of effort has been invested in early safety testing using the CSB™ approach during the past couple of years.[36] The goal is to satisfy the need for a predictive tool and understanding mechanisms of action early in the drug development process as part of "early safety testing" (see article in this volume by Giuliano et al.[39]). Cernostics, Inc. was spun off from Cellumen to focus efforts in patient sample profiling, and the analysis has been called Tissue Systems Biology (TSB™). It is my belief that the implementation of a systems biology perspective is a critical path for all fields of life science that use imaging methods.

SELECTED POSITIVE AND NEGATIVE OBSERVATIONS DURING THE 13-YEAR EVOLUTION OF HCS

Positive observations

The development of advanced analysis methods to reduce data for better understanding and to extract information from large data sets. HCS has been able to take advantage of the host of computational approaches developed initially for genomics and proteomics and even other fields but applied to cellular data.[15,16,19,23,40-42]

The attraction of academic physicists to the field to push the limits of resolution of light microscopes. There were great developments in light microscope imaging technologies from 1950 to 2000.[3,5] However, the excitement developed in the biological and biotechnology communities on the potential of advanced light microscope imaging methods encouraged physicists to take another look at pushing the boundaries on the limits of resolution in light microscopy. New super-resolution methods have emerged that could be incorporated into next-generation HCS platforms or used in conjunction with HCS,[43] based on the development of structured illumination.[44]

The use of systems biology approaches to explore significant biological questions, including heterogeneous cellular responses to perturbations. It has long been known that different states of cells give rise to subpopulations of responses. Until HCS, most cell-based assays used population averaging methods or investigators spent weeks and months using semiautomated research imaging approaches to collect only moderately large data sets.[45] The significance of these states, including oscillations, can best be investigated with physiologically relevant, attached cells using live-cell HCS coupled with informatics tools.[40,46]

Negative observations

The implementation of HCS in the pharmaceutical industry took even longer than I had expected. The "market" did not really start growing until about 2003. HCS was introduced when the industry was still focused on UHTS and combinatorial chemical libraries. Although there was a dramatic increase in the use of cell-based assays, it took a few more years than I expected to be fully accepted as a standard approach. In particular, it has taken even longer for the value of live-cell studies to be embraced.

The use of HCS as a quantitative tool continues to be limited by the absence of industry-wide standards. Unlike flow cytometry, HCS has only limited standards for irradiance at the specimen plane, fluorescence intensity, spectral overlap correction, and so on. Although basic research imaging has addressed many of these factors over the years,[5] there needs to be an industry-wide agreement on standards. Again, the societies can help to drive this, as they did for flow cytometry in the 1980s.

The limited integration of the various fluorescence and transmitted light modes of imaging into HCS platforms. The linkage between basic research imaging technologies and HCS has been discussed for many years, but the majority of HCS instruments have not incorporated even more powerful measurement tools such as ratio imaging, total internal reflection fluorescence, fluorescence lifetime, and other contrast methods.[3] There is great potential for combining modes of light microscopy into single instruments that can amplify the kind of data that can be acquired from the same samples.[47] As expected, academics are early in the combination of HCS with a variety of contrast methods.[47-51]

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WHERE ARE WE GOING?

HCS is continuing to evolve into many fields of life sciences. The academic community is fully engaged, and this will help to drive innovations from the technology to the biology. The technologies of the optical contrast methods, imaging technologies, informatics, bioinformatics, sample preparation, cells/tissues, and reagents will continue to evolve. The rebirth of light microscopy as a serious field in physics and engineering will continue to play an important role in these advances.[43]

It is my perspective that the biology will drive the development of the next-generation HCS platforms. I would expect that multimodal HCS systems[3,4,52] will be created to meet the demand for a range of spectroscopic measurements on the same samples in the same timeframe, as well as transmitted light contrast methods for some "label-free" measurements on the same sample. Novel and powerful reagents will continue to allow molecularly specific measurements.[7] Some of these developments will also benefit from further miniaturization of the samples from microplates to some form of a CellChip that will include on-board microfluidics and optics.

The biology will be very exciting over the next decade because we now have powerful platforms that can generate massive amounts of cell/tissue and even organism data that we can turn into systems information and knowledge. The most exciting thing to me is the feedback loop that can generate the data, extract the information, and create the knowledge that can then be fed into in silico models. The in silico models can then be queried, and more advanced questions can be asked and the process repeated. The field of cellomics/cytomics will be one of the next great challenges for the biomedical community.[53,54]

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It has been exciting taking part in the growth of both the academic side and the commercial side of light microscope imaging technologies over the past 30 years. I have been fortunate to work with some extraordinarily talented people during this period, including mentors, collaborators, graduate students, post-docs, and commercial colleagues. In particular, my academic mentor was the late Robert D. Allen, who, together with Shinya Inoue, influenced my scientific perspectives on the potential of studying living cells. Back in the early 1970s, when I was a young faculty member at Harvard, there were relatively few scientists exploring the use of light microscopes as quantitative tools. My students, post-docs, and I had a great time exploring technology and the biology. My later years at Carnegie Mellon University in the Center for Light Microscope Imaging and Biotechnology were exciting with great collaborations and interactions with faculty, including Alan Waggoner, Fred Lanni, Michel Nederlof, and Robert Murphy. The students and post-docs, too numerous to list, were central to our investigations. The 1980s and 1990s produced a host of outstanding academics and some companies pushing the limits of the technologies to explore the dynamics of cells and tissues. Leaving academia to start Cellomics was made less scary by the remarkable scientists and engineers who shared the vision of HCS. Terry Dunlay, Bert Gough, and Ken Giuliano require special mention. Recently, the excitement has continued with my colleagues at Cellumen and Cernostics harnessing the potential of cellular systems biology.

REFERENCES

1. Giuliano K, DeBiasio R, Dunlay T, Gough A, Volosky J, Zock J, et al: High-content screening: a new approach to easing key bottlenecks in the drug discovery process. *J Biomol Screen* 1997;2:249-259.
2. Schroeder K, Neagle B: FLIPR: a new instrument for accurate, high throughput optical screening. *J Biomol Screen* 1996;1:75-80.
3. Taylor D: Past, present and future of high content screening and the field of cellomics. In Taylor D, Haskins J, Giuliano K (eds): *Methods in Molecular Biology: Vol. 356. High Content Screening: A Powerful Approach to Systems Cell Biology and Drug Discovery*. Totowa, NJ: Humana, 2007:3-18.
4. Taylor D, Nederlof M, Lanni F, Waggoner A: The new vision of light microscopy. *Am Sci* 1992;80:322-335.
5. Wang, Y, Taylor, D (eds): *Fluorescence Microscopy of Living Cells in Culture: Parts A and B. Methods in Cell Biology*. Vols. 29 and 30. New York: Academic Press, 1989.
6. Taylor D, Wang Y: Fluorescently labeled molecules as probes of the structure and function of living cells. *Nature* 1980;284:405-410.
7. Giuliano K, Taylor D, Waggoner A: Reagents to measure and manipulate cell functions. In Taylor D, Haskins J, Giuliano K (eds): *Methods in Molecular Biology: Vol. 356. High Content Screening: A Powerful Approach to Systems Cell Biology and Drug Discovery*. Totowa, NJ: Humana, 2007:141-163.
8. Ding G, Fischer P, Boltz R, Schmidt J, Colaianne J, Gough A, et al: Characterization and quantitation of NF-KB nuclear translocation induced by interleukin-1 and tumor necrosis factor-α: development and use of a hight capacity fluorescence cytometric system. *J Biol Chem* 1998;273:28897-28905.
9. Conway B, Minor L, Xu J, Gunnet J, DeBiasio R, Giuliano K, et al: Quantitation of G-protein coupled receptor internalization using G-protein coupled receptor-green fluorescent protein conjugates with the ArrayScan high-content screening system. *J Biomol Screen* 1999;4:75-86.
10. Gough A, Johnston P: Requirements, features and performance of high content screening platforms. In Taylor D, Haskins J, Giuliano K (eds): *Methods in Molecular Biology: Vol. 356. High Content Screening: A Powerful Approach to Systems Cell Biology and Drug Discovery*. Totowa, NJ: Humana, 2007:41-61.
11. Taylor D, Woo E, Giuliano K: Real-time molecular and cellular analysis: the new frontier of drug discovery. *Curr Opin Biotechnol* 2001;12:75-81.
12. Giuliano K, Haskins J, Taylor D: Advances in high content screening for drug discovery. *Assay Drug Dev Technol* 2003;1:565-577.
13. Abraham V, Taylor D, Haskins J: High content screening applied to large-scale cell biology. *Trends Biotechnol* 2004;22:15-22.
14. Evans J, Matsudaira P: Linking microscopy and high content screening in large-scale biomedical research. In D Taylor, J Haskins, & K Giuliano (eds): *Methods in Molecular Biology: Vol. 356. High Content Screening: A Powerful Approach to Systems Cell Biology and Drug Discovery*. Totowa, NJ: Humana, 2007:33-38.
15. Perlman Z, Slack M, Feng Y, Mitchison T, Wu L, Altschuler S: Multidimensional drug profiling by automated microscopy. *Science* 2004;306:1194-1198.

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16. Boland M, Murphy R: A neural network classifier capable of recognizing the patterns of all major sub-cellular structures in fluorescence microscope images of Hela cells. *Bioinformatics* 2001;17:1213-1223.
17. Swedlow J, Goldberg I, Brauner E, Sorger P: Informatics and quantitative analysis in biological imaging. *Science* 2003;300:100-102.
18. Carpenter A: Image-based chemical screening. *Nat Chem Biol* 2007;3:461-465.
19. Hood L, Perlmutter R: The impact of systems approaches on biological problems in drug discovery. *Nat Biotechnol* 2004;22:1215-1217.
20. Klenk J: Characteristics and value of machine learning for imaging in high content screening. In Taylor D, Haskins J, Giuliano K (eds): *Methods in Molecular Biology: Vol. 356. High Content Screening: A Powerful Approach to Systems Cell Biology and Drug Discovery*. Totowa, NJ: Humana, 2007:83-94.
21. Tanaka M, Bateman R, Rauh D, Vaisberg E, Ramchandani S, Zhang C, et al: An unbiased cell morphology-based screen for new, biologically active small molecules. *PloS Biol* 2005;3:0764-0776.
22. Giuliano K, Chen Y, Taylor D: High content screening with siRNA optimizes a cell biological approach to drug discovery: defining the role of p53 activation in the cellular response to anticancer drugs. *J Biomol Screen* 2004;9:557-568.
23. Giuliano K, Wing C, Curran D, Day B, Kassick A, Lazo J, et al: Systems cell biology knowledge created from high content screening. *Assay Drug Dev Technol* 2005;3:501-514.
24. Taylor D, Haskins J, Giuliano K (eds): *Methods in Molecular Biology: Vol. 356: High Content Screening: A Powerful Approach to Systems Cell Biology and Drug Discovery*. Totowa, NJ: Humana, 2007.
25. Inglese J (ed): *Methods in Enzymology: Vol. 414. Measuring Biological Responses with Automated Microscopy*. New York: Elsevier, 2006.
26. Haney S (ed): *High Content Screening Science, Technology and Application*. New York: John Wiley, 2008.
27. Ekins S, Xu J (eds): *Drug Efficacy, Safety, and Biologics Discovery: Emerging Technologies and Tools*. Hoboken, NJ: John Wiley, 2009.
28. Tang F, Xu J (eds): A thematic issue on in vitro imaging. *Comb Chem High Throughput Screen* 2009; 12.
29. Lang P, Yeow K, Nichols A, Scheer A: Cellular imaging in drug discovery. *Nat Rev Drug Discov* 2006;5:343-356.
30. Bullen A: Microscopic imaging techniques for drug discovery. *Nat Rev Drug Discov* 2008;7:54-67.
31. Dragunow M: High content analysis in neuroscience. *Nat Rev Neurosci* 2008;9:779-788.
32. Feng Y, Mitschison T, Bender A, Young D, Tallarico J: Multi-parameter phenotypic profiling: using cellular effects to characterize small-molecule compounds. *Nat Rev Drug Discov* 2009;8:567-578.
33. Loo L, Wu L, Altschuler S: Image-based multivariate profiling of drug responses from single cells. *Nat Methods* 2007;4:445-453.
34. Thomas N: High-content screening: a decade of evolution. *J Biomol Screen* 2010;15:1-7.
35. Giuliano K, Premkuma D, Taylor D: Optimal characteristics of protein-protein interaction biosensors for cellular systems biology profiling. In Haney S (ed): *High Content Screening: Science, Technology and Applications*. New York: John Wiley, 2008:371-387.
36. Giuliano K, Johnston P, Gough A, Taylor D: Systems cell biology based on high content screening. *Methods Enzymol* 2006;414:601-619.
37. Vernetti L, Irwin W, Giuliano K, Gough A, Johnston P, Taylor D: Cellular systems biology applied to pre-clinical safety testing: a case study of CellCiphr® cytotoxicity profiling. In Ekins S, Xu J (eds): *Drug Efficacy, Safety, and Biologics Discovery*. New York: John Wiley, 2009:53-73.
38. Critchley-Thorne R, Miller S, Taylor D, Lingle W: Applications of cellular systems biology in breast cancer patient stratification and diagnostics. *Comb Chem High Throughput Screen* 2009;12:860-869.
39. Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA: Early safety assessment using cellular systems biology yields insights into mechanisms of action. *J Biomol Screen* 2010;15:783-797.
40. Loo L, Lin H, Steininger R, Wang Y, Wu L, Altschuler SJ: An approach for extensibly profiling the molecular states of cellular subpopulations. *Nat Methods* 2009;6:759-765.
41. Yin Z, Zhou X, Sun Y, Wong ST: Using iterative cluster merging with improved gap statistics to perform online phenotype discovery in the context of high throughput RNAi screens. *BMC Bioinform* 2008;9:264.
42. Young D, Bender A, Hoyt J, McWhinnie E, Chirn GW, Tao CY, et al: Integrating high-content screening and ligand-target prediction to identify mechanism of action. *Nat Chem Biol* 2008;4:59-68.
43. Milestones in light microscopy supplement [Online]. Retrieved from www.nature.com/milestones/light-microscopy
44. Bailey B, Farkas D, Taylor D, Lanni F: Enhancement of axial resolution in fluorescence microscopy by standing-wave excitation. *Nature* 1993;366:44-48.
45. Bright G, Whitaker J, Haugland R, Taylor D: Heterogeneity of the changes in cytoplasmic pH upon serum stimulation of quiescent fibroblasts. *J Cell Physiol* 1989;141:410-419.
46. Slack M, Martinez E, Wu L, Altschuler S: Characterizing heterogeneous cellular responses to perturbations. *PNAS* 2008;105:19306-19311.
47. Collins J: FLIM for high content-thinking outside of the box. *Screen Trends Drug Discov* 2008;3:14-16.
48. Paran Y, Ilian M, Kashman Y, Goldstein S, Liron Y, Geiger B, et al: High throughput screening of cellular features using high-resolution light microscopy: application for profiling drug effects on cell adhesion. *J Struct Biol* 2007;158:233-243.
49. Vizeacoumar F, Chong Y, Boone C, Andrews B: A picture is worth a thousand words: genomics to phenomics in the yeast *Saccharomyces cerevisiae*. *FEBS Lett* 2009;583:1656-1661.
50. Barber P, Pierce G, Ameer-Beg S, Matthews D, Carlin L, Keppler M, et al: Towards high throughput FLIM for protein-protein interaction screening of live cells and tissue microarrays. In *2008 5th IEEE International Symposium on Biomedical Imaging: From Nano to Macro*. Washington, DC: IEEE, 2008:356-359.
51. Talbot C, McGinty J, Grant D, McGhee E, Owen D, Zhang W, et al: High speed unsupervised fluorescence lifetime imaging confocal multiwall plate reader for high content analysis. *J Biophotonics* 2008;1:514-521.
52. Giuliano K, Nederlof M, DeBiasio R, Lanni F, Waggoner A, Taylor D: Multi-mode light microscopy. In Herman B, Jacobson K (eds): *Optical Microscopy for Biology*. New York: Wiley-Liss, 1990:543-557.
53. Satulovsky J, Lui R, Wang Y-L: Exploring the control circuit of cell migration by mathematical modeling. *Biophys J* 2008;94:3671-3683.
54. Zhao T, Murphy R: Automated learning of generative models for subcellular location: building blocks for systems biology. *Cytometry* 2007;71A:78-99.

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