Miniaturized HTS technologies – uHTS

Julian Wölcke and Dirk Ullmann

The transition from slow, manual, low-throughput screening to industrialized robotic ultra-high throughput screening (uHTS) in the past few years has made it possible to screen hundreds of thousands of chemical entities against a biological target in a short time-frame. The need to minimize the cost of screening has been addressed primarily by reducing the volume of sample to be screened. This, in turn, has resulted in the miniaturization of HTS technology as a whole. Miniaturization requires new technologies and strategies for compound handling, assay development, assay adaptation, liquid handling and automation in addition to refinement of the technologies used for detection systems and data management. This review summarizes current trends in the field of uHTS and illustrates the technological developments that are necessary to enable the routine application of miniaturized uHTS systems within an industrial environment.

Julian Wölcke and *Dirk Ullmann
Drug Discovery Services
Screening Operations
Evotec OAI
Schnackenburgallee 114
D-22525 Hamburg
Germany
tel: +49 405 608 1247
fax: +49 405 608 1222
e-mail: dirk.ullmann@evotecoai.com

▼ As HTS progresses into the next century, a concomitant consolidation of the complex synergy between associated developments in chemistry, biology, engineering and informatics for lead discovery is necessary. It is anticipated that, in addition to providing innovative solutions to technological challenges, this consolidation must also enable incorporation of uHTS technologies into the infrastructure of the pharmaceutical industry. It has been estimated that industrial screening demands will require the number of new chemical entities introduced per year to be tripled, necessitating a threefold increase in the speed of current screening technology[1]. Miniaturization provides a key means of keeping pace with genomics because it enables proportionately more targets and samples to be screened per unit time[2]. Because of the small amounts of compounds and reagents used in a miniaturized system, this increase in screening speed can be achieved without an increase in associated R&D costs[3–9]. As a result of the benefits outlined previously, miniaturization forms a key foundation of screening philosophy. Combining miniaturized technology with developments in automation, sensitive signal-detection, plate formats, automated compound-delivery and data management results in highly efficient, and cost-effective, integrated miniaturized uHTS systems.

This review will concentrate on recent major developments that have occurred in the uHTS arena over the past few years. Adaption of assay designs for uHTS running conditions will also be discussed.

Assay miniaturization

Assays themselves present a significant demand on uHTS systems. Established biochemical assays must be readily adaptable to miniaturized formats to shorten overall screening cycle-time. Lab-on-a-chip and microscale total-analysis systems are highly miniaturized. Such systems promise assay volumes at the picoliter level and throughputs that will easily exceed 100,000 assays per day[10].

In addition to increasing assay throughput by incorporating low volume, high density formats, further improvement can be achieved through the use of multiplexing strategies. Multiplexing involves the detection of multiple screening-parameters simultaneously or in rapid sequence[6]; such parameters might include the fluorescence polarization, intensity, lifetime and emission wavelength of a single or multiple species. In many target classes, it is now possible to design assay systems that involve mixing the components, incubating to a suitable end-point or equilibrium and measuring a detection signal[11–14]. This homogeneous 'mix and measure' type of assay is ideal for HTS. Functional cellular assays in miniaturized format are increasing in importance as primary screening assays. Although cell-based assays using reporter genes have proved effective as an HTS format, detecting more immediate responses to target-protein activation provides several advantages, including shorter assay duration and fewer

1359-6446/01/$ – see front matter PII: S1359-6446(01)01807-4

Table 1. Advantages and disadvantages of miniaturized uHTS

Miniaturized uHTS	Positive	Negative
Key Features		
Compound logistics	Saving of precious compounds High-density storage possible Potential for automated fractionation of natural extracts into screening plates	Reformatting step from 96, 384 to ≥1536-well plates necessary
Assay miniaturization	Saving precious target or ligand	Necessary effort because of altered surface:volume ratio and evaporation issues (adsorption effects) in high-density formats Solution: Benchtop workstations for assay development, consisting of the same liquid-handling hardware as screening devices necessary for assay compatibility tests
Plate formats	High compound density (less process steps, more parallelization) allows enhanced throughput 1536-well plates already compatible with many systems Plates of 2080 and 3456 wells already routinely applied	Increased demand on plate manufacturing Precise plate adjustment in dispensing and reading systems necessary
Liquid handling	Precise and reliable nanoliter handling with piezoelectric drop-on-demand systems 96 or 384-Well parallel dispensing enhances velocity of assay assembly	Cell handling difficult, but possible
Detection systems	Confocal detection independent of miniaturization. Imaging technologies applicable to fast 1536-well reading Fast 96 or 384-well parallel reading	Restricted to fluorescence or luminescence based readouts Macroscopic fluorescence sensitive to miniaturization
Automation	Fully automated screening platforms (integrated or modular) available for screening in 1536, 2080 or 3456-well plates	Sealed humid atmosphere as protection against evaporation necessary
Data management	Automation of data handling is worthwhile because of high data amounts	Increased efforts in database administration and data mining

false-positives from non-specific interactions[15]. Recent advances in miniaturization technology and molecular biology have made it possible to monitor, for example, the presence of second messengers (Ca^{2+}, cAMP, inositol triphosphate), phosphorylation of intermediate signaling molecules or subcellular translocation.

Assay miniaturization is the process of establishing optimal assay conditions for the microliter volume range that is necessary for the screening of high-density-well plates, thus minimizing reagent consumption and reducing storage capacity (Table 1). Several issues need to be tackled during assay miniaturization:

- Appropriate and accurate liquid handling (e.g. dispensing of cells through narrow-bore pipettes presents particular difficulties);
- Minimizing evaporation effects;
- Ensuring comparable assay sensitivity (dynamic range, binding constant K_d, IC_{50}) and screening statistics;
- Tackling the increased surface:volume ratio, which increases adsorption effects;
- Reproducing the conditions to be encountered on the HTS system as closely as possible (i.e. tackling issues such as reagent stability, kinetics of enzyme reactions and sedimentation and viability of cells); and
- Using full high-density-plate layouts in assay development and assay miniaturization.

Solutions to overcome these problems at the nanoscale level include altering the concentration and/or the order of addition of assay reagents (e.g. by adding 'sticky' reagents last) and adding detergents that reduce non-specific binding

in a typical concentration range between 0.01% and 0.5%. Automated assay optimization (AAO), which takes advantage from the statistical design of experiments (DOE), is a key method in the reduction of assay parameters and is ideal for application to high-density-plate formats. If used properly, AAO enables uHTS laboratories to reduce assay-optimization timelines and to optimize 'throw away' assays that would not be a subject of a screening run under usual conditions.

The translation of assay protocols from assay development via assay miniaturization to the HTS platform is a challenge that must not be underestimated. The use of benchtop workstations with hardware components identical to those installed within the uHTS system is the key factor for running a uHTS factory successfully.

Trends in plate formats

Currently, 96- and 384-well microplates still serve as a standard format for the majority of all HTS procedures[16]. In these plates, an overall assay volume of ~100 µl is used. Thus, at least 10 l of assay solution is necessary to screen 100,000 compounds for activity. Assuming a target concentration of 10 nM, a minimum amount of 100 nmol would be needed for an HTS run (i.e. 10 mg of a target with a MW of 100 kDa). Moreover, for pharmaceutical screening purposes, limited amounts of compounds need to be tested in many different assays. Therefore, many HTS units are moving towards high-density plates to overcome these limitations and to save compounds. From a technological point of view, these sample arrays can be handled more rapidly because several processes can be carried out in parallel and the number of process steps is reduced. The 384-well format plate is now beginning to supersede earlier plate formats for both storage of compounds and screening[17]. This type of microtiter plate (MTP) can already be handled by nearly all available HTS devices. An overall assay volume in the range of 10–50 µl is used in this type of carrier.

In general, an assay is regarded as miniaturized if the test volume is restricted to ≤5 µl, leading to a saving of compounds and reagents of at least 20-fold compared with the 96-well format. This target can be realised through the use of plates with 1536 wells or more. Currently, many companies offering plates, detection systems or liquid handling for uHTS are dealing with the transition to this type of plate. The higher the density of the plate format, the more stringent the requirements for flatness, rigidity, symmetry, well-depth and uniformity become. Consequently, until now, only a few providers offer high quality 1536-well plates for miniaturized assays (e.g. Greiner; Frickenhausen, Germany; Corning; Acton, MA, USA; Nunc, Rochester, NY, USA)[18,19]. Beyond the 1 µl assay scale, limitations concerning assay evaporation, poor surface:volume ratios and liquid handling become apparent. Plates of 2080 (NanoCarrier™ 2080; Evotec OAI, Hamburg, Germany) or 3456 wells (NanoWell™ Assay Plate, Aurora Biosciences, San Diego, CA, USA) are already used routinely in miniaturized uHTS. Even plates of 9600 wells[20] have been described for screening at the ≤1 µl scale. A 2080-well format, for example, allows the distribution of 1536 compounds, four lines of wells that contain assay controls, and a water rim for protection against assay evaporation.

In the case of cellular assay systems, the amount of miniaturization that can be undertaken is limited because of variation between cells, which dominates variation between samples at concentrations <100–1,000 cells per well, and cell viability is poor in sub-microliter liquid handling. Both of these problems present significant technological challenges that will be difficult to overcome.

Chip technologies and microfluidic devices, known as 'lab-on-a-chip', offer an insight into future test systems that are being developed for specific applications[20–23]. Moreover, prototypes of sample arrays containing >5000 wells on 10 cm petri dishes have been produced by applying photolitographic methods[24].

Liquid handling

The miniaturization of assays for uHTS and the enhancement of throughput require devices for fast liquid-handling in at least the nanoliter range. This requirement results from the fact that most assays require multiple reagent addition to result in a total assay volume of 1–5 µl. Because the assays to be screened are usually developed off-line, these liquid-handling devices need to be integrated into both benchtop and screening systems to enable assay development in directly comparable formats. Three major groups of pipetting and dispensing techniques are currently available for sub-microliter liquid-handling in miniaturized uHTS: (air)-displacement, pintool (liquid transfer based on metal pins – discussed later) and drop-on-demand systems[25,26].

(Air)-displacement systems incorporate pump sets, containing, for example, tiny pistons, that generate a pressure and lead to a displacement of air or system liquid in a dispensing unit. Most systems work in a non-contact mode. They allow fast and precise handling (standard deviations <10 %) of volumes between 0.5 and 100 µl and are, therefore, used primarily for assays in the range of between 5 and 100 µl. There are, however, products of this type available that allow nanoliter volumes to be dispensed for miniaturized uHTS (i.e. synQUAD™; Cartesian Technologies, Irvine, CA, USA and BlueBIRD™, Pharmacopeia, Princeton,

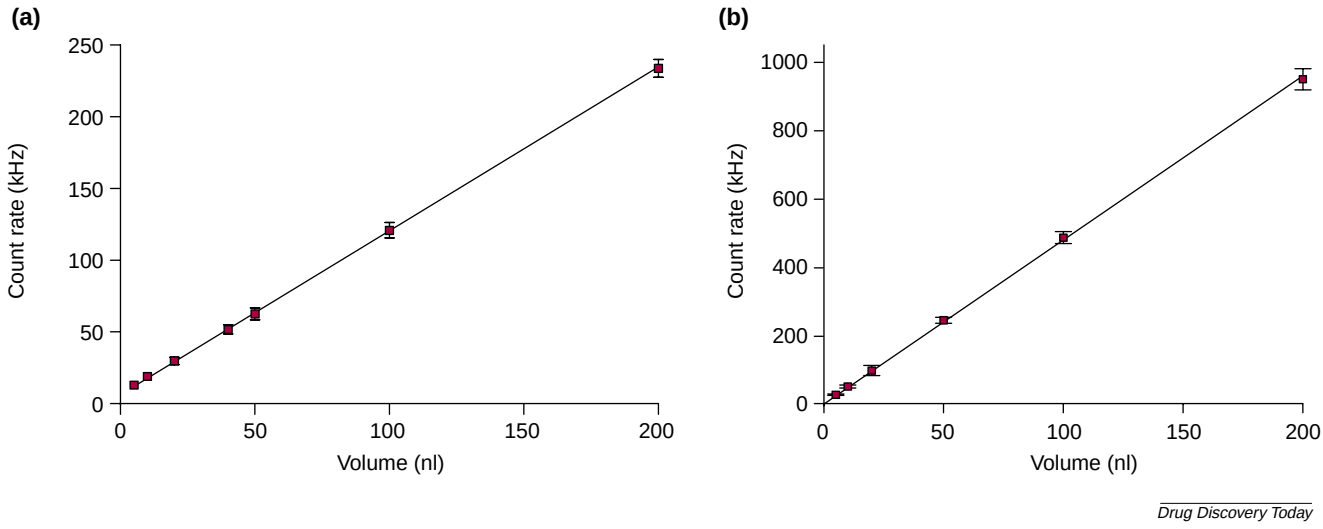


Figure 1. (a) The accuracy of low-volume dispensing at nanoliter (nanoliter) level based on the piezoelectric drop-on-demand system. A 20 nM dye solution was dispensed into a 2080 NanoCarrier™ (Evotec OAI, Hamburg). **(b)** The accuracy of low-volume pipetting (aspiration and dispensing) at the nanoliter level based on the piezoelectric drop-on-demand system. A 100 nM dye solution was pipetted into a 2080 NanoCarrier™, resulting in a correlation coefficient of 0.99.

NJ, USA[27]). The synQUAD™ dispensers are reported to allow the distribution of vital cells and so they can even be applied for miniaturized uHTS of cellular assay types (http://www/cartesiantech.com/ hts_products.htm).

Pintool systems are based on capillary and surface tension forces[26,28]. Tiny, metal needles are dipped into the liquid to be transferred to the assay. The amount of liquid taken up depends on the design of the pin. Either a solid pin, holding a small sample drop on its tip, or a split or quill pin, holding the sample within a slot, is used. The split or quill pin allows multiple spottings per loading. Both types of pins transfer liquids in the picoliter–nanoliter range and are thus, in principle, well suited for miniaturized uHTS. However, the major disadvantage of these techniques is the high spot-to-spot standard deviations of 10–30%. This variation is caused by the dependence of the transferred volume on the surface chemistry of the liquid and by the variations of the pin geometry. Some pintool systems are commercially available for microarray technology (i.e. PinArray™; Cartesian Technologies; BioGrid, BioRobotics, Cambridge, UK).

The drop-on-demand systems were developed according to the design of inkjet systems already used in the printing industry. Existing inkjet technology was well suited for adaptation to the dispensing of reagents at nanoliter level[26,29,30]. In principle, a liquid stream of reagent at high velocity is created and then forced through a small aperture, resulting in the ejection of small drops. This can be achieved by piezoelectric, syringe–solenoid or thermal systems[31]. In the piezoelectric systems, a voltage is applied across a piezoelectric ceramic actuator, causing a change of the actuator's shape and squeezing a capillary that contains assay reagent. Consequently, the liquid is then ejected in the form of a drop. The same result can be achieved by a syringe–solenoid actuated system. A syringe pump creates a pressure on a reservoir containing liquid. A solenoid valve opens for several microseconds and drops of assay solution are ejected resulting in a pressure release. Usually, the droplet size is found to be in the nanoliter–microliter range. The piezoelectric system is capable of creating between one hundred and several thousand drops per second in the picoliter–nanoliter range, enabling high accuracy for assay systems at the 1 µl level. The accuracy of low-volume dispensing that can be achieved with such systems is shown in Fig. 1a and 1b. The piezoelectric driven pipettes and dispensers can be produced using 'bulk silicon micromachining', which guarantees uniformity and enables the microproduction of the capillaries. Dispensers and pipettes for miniaturized uHTS are commercially available (ActiveTip M™, Tecan, Hombrechtikon, Switzerland; PiezoTip™, Packard Instruments, Meriden, CT, USA; and Micropumps TK- and TMP-Type, Evotec OAI, Hamburg, Germany). The piezoelectric system is sensitive to gas bubbles and precipitations. These effects can be overcome by the so-called 'T-channel' concept[32]**:** in a washing mode, a positive pressure on a rinsing fluid is created and leads to the positive displacement of bubbles or precipitates in the pump chamber or capillary. The combination of piezoelectric and pressure-driven systems is also applied in

high-accuracy aspirate-dispense modes, in which a liquid is aspirated, driven by the negative pressure through a syringe pump, and dispensed with high accuracy in a piezoelectric mode. This aspirate-dispense mode can be used for the reformatting of compounds or reagents in uHTS procedures of miniaturized assays. Standard deviations of <10% can be achieved for volumes as small as 10 nl, as demonstrated in Fig. 1b[29].

Parallel dispensing is usually applied to 96- to 1536-well uHTS to enhance the speed of assay assembly. In contrast to 96- and 384-well parallel dispensing, no single-step 1536-well parallel dispensing has been reported yet. However, many of the 96- and 384-well dispensers can be used to process 1536-well plates in 16 or four steps, respectively (Tango™, Robbins Scientific, Sunnyvale, CA, USA; CyBi™ Well, CyBio, Jena, Germany[33]; ProSys 96, Cartesian Technologies; PlateMate, Matrix Technologies, Hudson, NH, USA).

Alternative dispensing principles are currently in development, but are generally far from being incorporated into routine HTS procedures. The 'shock principle', for example, is based on the generation of mechanic shocks upon a cavity containing a liquid, this in turn leads to the ejection of droplets from a nozzle[34]. In microfluidic 'lab-on-a-chip' devices, fluids are moved through microscopic channels by non-mechanical, electrokinetic processes such as electrophoresis or electro-osmosis[35]. Such systems are capable of sorting and manipulating single, live, suspended cells[21,22,35–36].

Detection systems

Apart from its suitability for miniaturization and automation, additional desirable characteristics of uHTS assay technology include robust signal generation and statistics. Although conventional, and imaging, scintillation proximity assays (SPAs) have undoubtedly increased the future use of radiolabel-based approaches, most new assay technologies incorporate alternative, non-radioactive readouts, primarily fluorescence and luminescence[37]. Generally, fluorescent-readout technologies can be divided into: those that monitor the time- and assay-volume-weighted ensemble average of the fluorescence emission from a well (macroscopic approach, cannot differentiate between differences or lifetimes of fluorescent molecules); and those that monitor the fluorescence properties of the individual molecules within the assay-well in a stochastic fashion (single-molecule approach). The former techniques include most of those currently employed in HTS, such as prompt fluorescence intensity (FLINT), fluorescence resonance energy transfer (FRET)[38], time-resolved energy transfer (TRET)[39,40] and fluorescence anisotropy (FA)[41], whereas single-molecule techniques are exemplified by fluorescence correlation spectroscopy (FCS)[42], fluorescence-intensity distribution analysis (FIDA)[43] and related multi-dimensional techniques[44]. Table 2 shows current hardware platforms that are suitable for miniaturized uHTS and that are capable of handling plate formats of >1536 wells.

Because there are several articles that review homogeneous fluorescent readouts[45,46], beside imaging technologies[47], confocal fluorescence is one principal readout technology that is ideally suited for miniaturized uHTS. Confocal readouts are effectively insensitive to miniaturization because of the small size of the confocal detection volume (10^{-15} l). Confocal techniques report on the properties and number of the individual fluorescence molecules within the confocal volume, in contrast to the time-, volume- and concentration-averaged signal output from the other macroscopic fluorescence techniques mentioned previously. The improvements in confocal optics have reduced read-times to one, or a few, seconds per well for many types of assays; thus, such detection systems are capable of delivering the throughputs necessary for uHTS (FCS+*plus* detection system, Evotec OAI; ConfoCor2, Analytikjena AG, Jena, Germany). FIDA enables the non-radioactive screening of membrane-receptor assays; free-ligand and ligand-accumulated on receptor-bearing membrane vesicles can be distinguished on the basis of their particle brightness (i.e. an additive intensity effect is observed dependent of ligand-binding)[48]. Multi-dimensional confocal detection technologies [for example, 2-dimensional (2D)-FIDA] are tools that can distinguish fluorescent species with different specific polarization ratios or are able to determine concentrations and specific brightness values of fluorescent species corresponding to multiple wavelength emissions. For example, the 2D-FIDA method can be applied to investigate protein-tyrosine-kinase assays using a generic assay principle[6].

Future trends in detection technologies are likely to include the use of laser-scanning systems coupled with multi-parameter discrimination algorithms (ACUMEN, The Technology Partnership, Royston, UK) and the introduction of additional fluorescence readouts, for example, fluorescence lifetime[49,50]. Furthermore, the introduction of multiphoton excitation systems to uHTS systems will permit confocal excitation without the need for a pinhole, along with the added benefit of reduced phototoxicity of the longer wavelength light required for multiphoton excitation[51].

Automation

One necessary prerequisite for the generation of millions of data points of compound activity per year in the

Table 2. Detection systems for miniaturized uHTS

System	Features	Manufacturer
ACUMEN	Laser-scanning microscope Point scanner Homogeneous + cell-based assays	The Technology Partnership, Royston, UK
AlphaQuest-HTS	Optimized for highly sensitive detection of AlphaScreen reagents	Packard Instrument Company, Meriden, CT, USA
CLIPR™	Luminometer, CCD camera Cell-based assays, SPA	Molecular Devices, Sunnyvale, CA, USA
CyBiTM-Lumax 1536 S	Luminometer	CyBio AG, Jena, Germany
FCS + plus Reader	Multi-detection confocal reader Free formats (1536/2080 NanoCarriers)	Evotec OAI, Hamburg, Germany
FLUO-/POLARstar Galaxy	Multi-detection reader (5 modes), FLINT and TRET for 1536-well plates	BMG Labtechnologies GmbH, Offenburg, Germany
LEADSeeker	Microplate imager Cooled CCD camera Radiometric + fluorimetric toolboxes, well- + cell-analyzer	Amersham Pharmacia Biotech, Uppsala, Sweden
LJL Acquest	Multimode detection system: FLINT, polarization, TR-FRET, luminescence	Molecular Devices, Sunnyvale, CA, USA
Multimode Reader	Lens array containing 96 parallel micro-objectives: 1,536 wells in 16 steps	Carl Zeiss Jena, Jena, Germany
SPECTRAFLUOR Plus	Dual optical channels, fast switch top-bottom reading, fluorescence, absorbance, luminescence	TECAN, Maennedorf, Switzerland
ULTRA Reader	Multimode detection system, no changes in optical system, fluorescence, TRET, polarization, luminescence, cell-based assay functionality	TECAN, Maennedorf, Switzerland
Victor^2	Multilabel counter (10 modes) 1536 Fluorescence Optional dispencer module	Perkin Elmer Life Sciences, Wallac Oy, Turku, Finland
Victor^2-V	Multilabel counter FLINT, TRET (LANCE), polarization, luminescence	Perkin Elmer Life Sciences, Wallac Oy, Turku, Finland
ViewLux	Microplate imager Cooled CCD camera, TRET (DELFIA, LANCE)	Perkin Elmer Life Sciences, Wallac Oy, Turku, Finland

Abbreviations: CCD, charged coupled device; SPA, scintillation proximity assays; FLINT, fluorescence intensity; TRET, time-resolved energy transfer; TR-FRET, time-resolved fluorescence resonance energy transfer.

pharmaceutical lead identification process is the development of robust lab automation. These automation concepts are either based on stand-alone workstations, or alternatively on modular workstations or integrated screening. Modular systems are less suited for miniaturized uHTS (no control of evaporation and temperature), but permit a high degree of flexibility in infrastructure design, a factor that is of great importance for new assay-technology development, but of less significance for uHTS process runs. Automation platforms with an open architecture that enables incorporation of different modules from one supplier also offer high flexibility, because different devices can be reassembled, depending on the demands of the specific assay to be screened[52,53]. Such systems are available in 384 (Allegro™, Zymark, Hopkinton, MA, USA; Robolab®, Robocon, Vienna, Austria) or even 1536-well miniaturized

uHTS formats (CyBi™-Screen-machine, CyBio; Zeiss UHTS, Carl Zeiss Jena, Jena, Germany; Robolab®-1536, Robocon). Additional modules from different suppliers can potentially be used in conjunction with such open-architecture systems. However, combining devices from independent product lines in this way can result in software integration problems, especially at transfer positions and at the level of scheduling the overall screening process.

The flexibility of the integrated screening concept with regard to a specific assay to be screened is as high as that of the automated modular system, but here, the different modules do not need to be reassembled physically. The integrated systems listed below enable fully automated miniaturized uHTS and most of them are already used for routine screening: EVOscreen™ (Evotec OAI)[54,55]; UHTSS™ (Aurora Biosciences; San Diego, CA, USA)[56]; uHTS platform (Pharmacopeia, Princeton, NJ, USA)[57]; and Asset (The Automation Partnership, Royston, UK)[58]. In each of these systems, the number and sequence of process steps is solely defined within the software. Highly complex assays can be run and scheduled dynamically to optimize assay conditions and throughput. A sealed system that maintains a humid atmosphere on assay-assembly stations and readers in miniaturized HTS can be realized as part of a fully automated screening-platform. Similarly to modular systems, the integration of several different detectors for specific readouts in the fully integrated HTS systems is possible, but needs to be harmonized in terms of software. Through the application of powerful readers that are capable of multiplexing, only a minimum reader re-assembly would be necessary.

Data management

Screening of many thousand compounds per day necessitates the development of specially designed data analysis and data storage systems that fulfill several criteria. The analysis environment must offer simultaneous access to several different data sources, and be capable of generating visual data displays that promote multidimensional reasoning. Such software should also have sufficient flexibility to lend itself to the publishing of data in a collaborative work-group environment (Activity Base™, ID Business Solutions, Guildford, UK; Assay Explorer, MDL Information Systems, San Leandro, CA, USA) (Fig. 2)[59–63].

Every screening task must pass through an online data validation and quality control step when a screening plate is read. This requires a flexible working infrastructure that makes it possible to evaluate different readouts based on positive and negative control wells, and the calculation of screening-results, in terms of inhibition or activation values. In addition, such a tool has to enable the calculation of statistical key parameters [e.g. Z′, Z, signal-to-background (S:B) and signal-to-noise (S:N)] and prepare the rescreening decision immediately based on, for example, Z′ thresholds (Fig. 3)[64].

The software must also be able to re-map the primary screening data from the screening plates to the original master-plate formats. In summary, on-line quality control (QC) is a systematic way of surveying entire plates, batches, runs and even screens for the trends and outlying data points that can indicate biological or instrumentation problems.

Although the data volume and speed of data acquisition both decrease at the lead optimization stage, a concomitant increase in data complexity is introduced with the inclusion of *in vivo* assays, computational and structure–activity relationship assessments, medicinal and physicochemical studies, and data from the development of compound libraries. The diversity of screening data also increases with the introduction of high-content or high-information screening systems, most commonly based on imaging systems. Enabling full exploitation of such complex, high density uHTS data requires meta-data structures, such as extensible markup language (XML). Meta-data structures describe underlying data, providing context and the ability to associate screens according to common attributes. Visualization tools can be used to great effect during uHTS for QC, database navigation and data-mining (Spotfire®, Spotfire, Cambridge, MA, USA).

Current emerging technologies, such as Internet-platform databases, new standards for data interchange and the storage and the introduction of alerting tools and intelligent agents will have a significant impact on screening over the next years. Further new directions in HTS data analysis include statistical analysis techniques that link primary screening results to hit confirmation and validation[65].

Future trends

Miniaturization and automation are required to reduce the costs of the traditional screening approach relative to the alternative strategy of compound pooling. Although the pooling of 3–10 compounds per bioassay has been used to quickly and efficiently assay large compound sets, the primary disadvantage of this technique is the need for subsequent deconvolution of positive read-outs, and the potential for masking of the activity of one compound by that of another. In secondary screening, multifunctional testing for parameters such as selectivity, toxicity and dose-response is conducted. By combining miniaturization, automation and multiple detection-modes, this process can be developed further within a uHTS framework.

Further emerging trends in miniaturized uHTS include the HTS of single nucleotide polymorphisms (SNPs)[66].

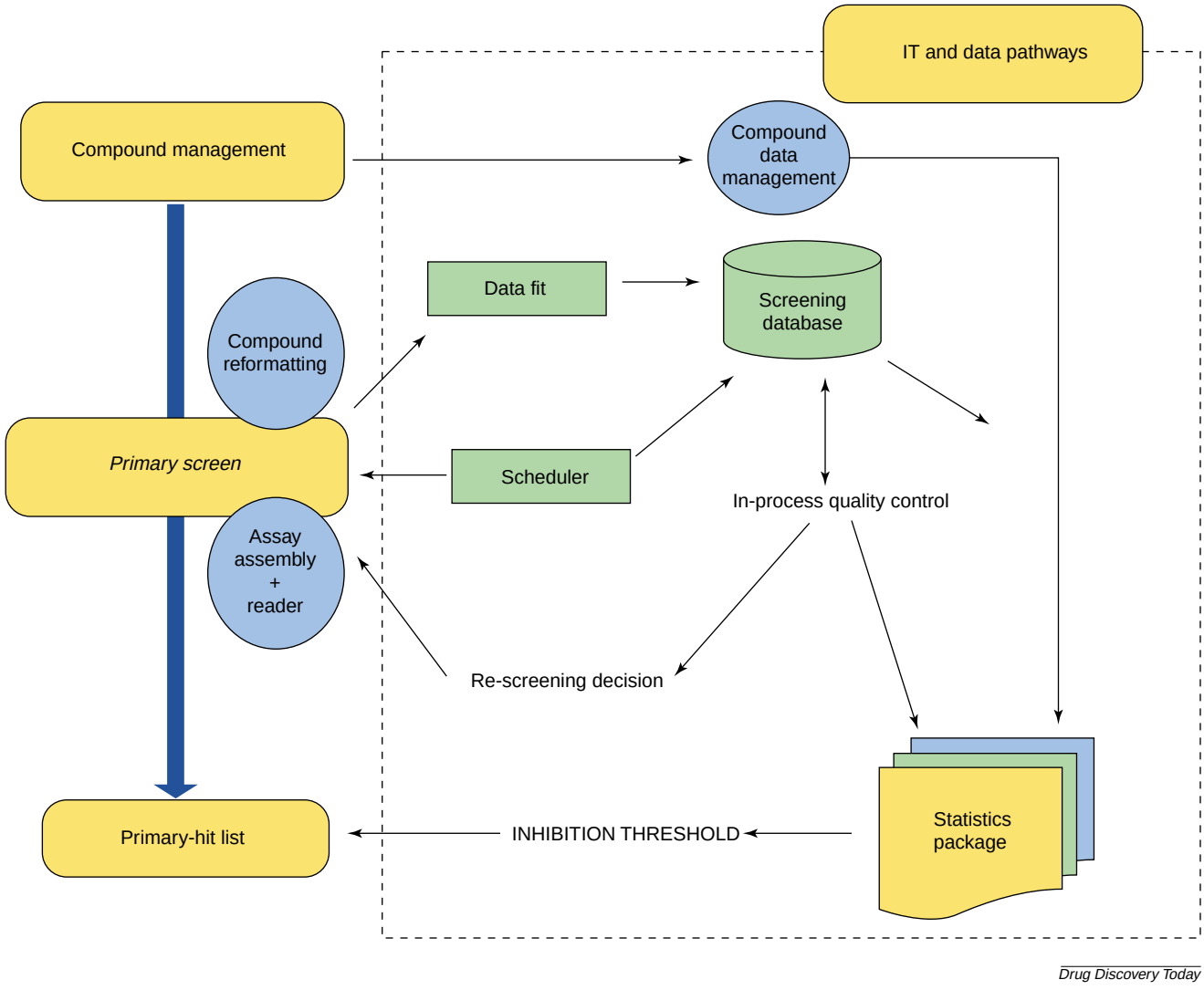


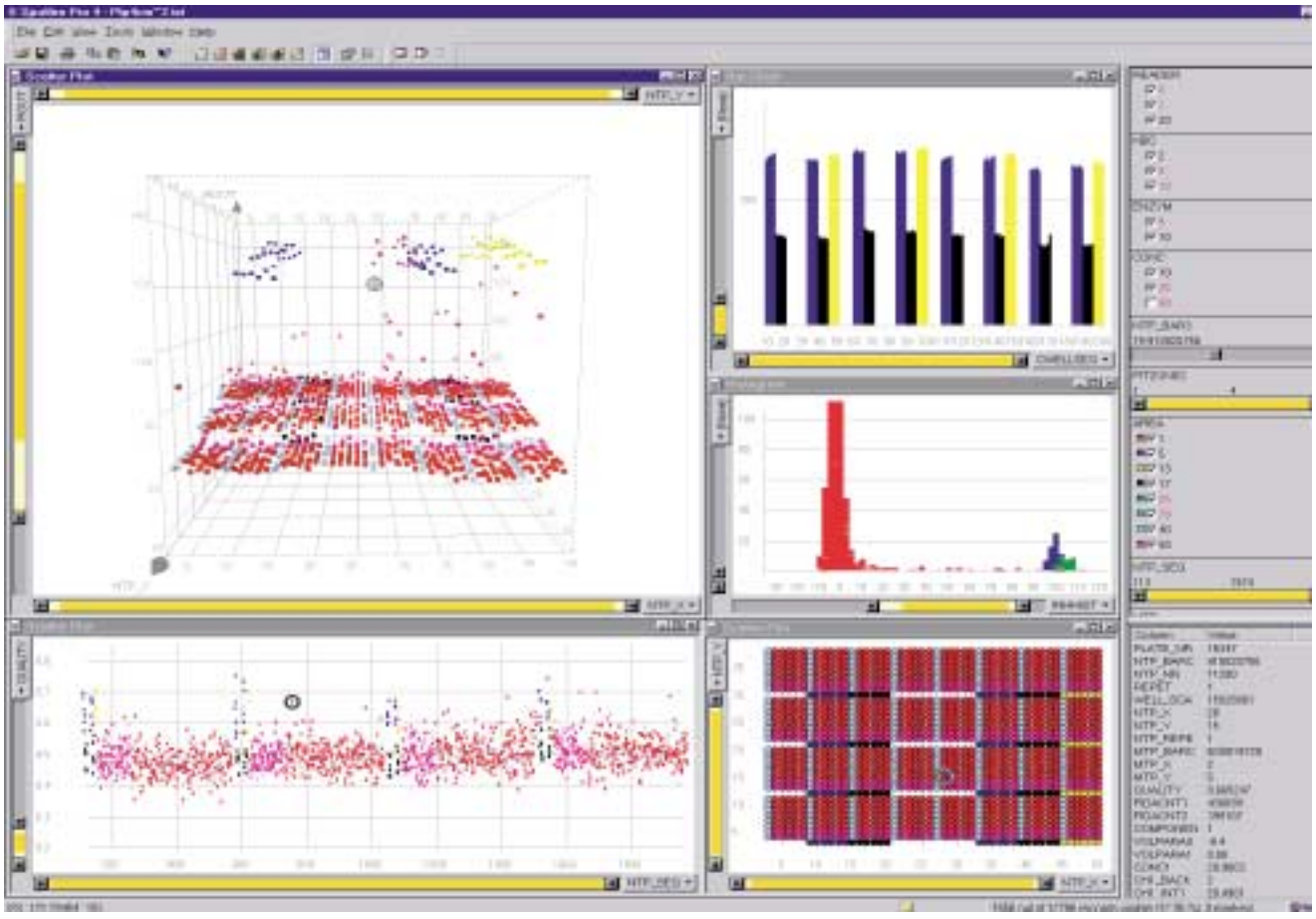
Figure 2. An overall screening process involves several levels of data management: scheduling of the screening devices, handling of compound data and management of screening results.

Genotyping, the process of creating associations between SNPs and disease, is a key objective for applying information gained from sequencing of the human genome.

In conclusion, the need to simultaneously shorten the drug discovery process and allow millions of new compounds to be tested against hundreds of new targets has led to a complete re-evaluation of screening technology. Miniaturization and automation cannot be avoided if screening is to remain economical. A maximum amount of information must be gleaned from each assay. This means multiple read-out modes must be used to assess a wide variety of parameters in a single sample. Because *in vitro* assays lend themselves to miniaturization and integration into a HTS format, new compounds can be directly tested for biological significance, and the data obtained can be used to help design the next iteration of lead optimization. Finally, virtual screening[67], which encompasses a variety of computational techniques that allow chemists to reduce a huge virtual library to a more manageable size will be a tool of the future that, in combination with 'wet' screening, leads to the evaluation of larger number of compounds[68].

Acknowledgements

We would like to thank Hans H. Grünhagen, Axel Ehlert, Michael Busch and Martina Leimkühler at Evotec OAI for helpful discussions during the preparation of the manuscript, and Timm Jessen (Evotec OAI) for his continuous scientific support. We are very grateful to Mary Cole (Evotec OAI) for reviewing this paper and providing advice and helpful suggestions.



Drug Discovery Today

Figure 3. A 'screengrab' showing the on-line quality control in uHTS data evaluation. The view comprises, anticlockwise from bottom-right: a plate layout on a 2080-well NanoCarrier™ (Evotec OAI, Hamburg, Germany). Red: combichem compound data; purple: natural product data; blue: high control; black: low controls; yellow: high control 2. (There are four control lanes on one NanoCarrier™ to correct for drifts in both x- and y-direction.) 2D and 3D plate views; and plate histograms.

References

1 Drews, J. (1996) *Innovation Deficit In The Pharmaceutical Industry*. Drug Information Association

2 Dunn, D. *et al.* (2000) Ultra-high throughput screen of two-million-member combinatorial compound collection in a miniaturized, 1536-well assay format. *J. Biomol. Screen.* 5, 177–187

3 Taylor, P. *et al.* (2000) Automated assay optimization with integrated statistics and smart robotics. *J. Biomol. Screen.* 5, 213–225

4 Bosse, R. *et al.* (2000) Miniaturizing screening: how low can we go today? *Drug Discov. Today* (HTS Suppl.), S42–S47

5 Hertzberg, R.P. and Pope, A.J. (2000) High-throughput screening: new technology for the 21st century. *Curr. Opin. Chem. Biol.* 4, 445–451

6 Ullmann, D. *et al.* (2000) Fluorescence correlation spectroscopy-based screening technology. *Inn. Pharm. Tech.* 99, 30–40

7 Harrison, W. (1998) Changes in scale in automated pharmaceutical research. *Drug Discov. Today* 3, 343–349

8 Sills, M.A. (1998) Future considerations in HTS: the acute effect of chronic dilemmas. *Drug Discov. Today* 3, 304–312

9 Divers, M. (1999) What is the future of high throughput screening? *J. Biomol. Screen.* 4, 177–178

10 Bellavance, L. *et al.* (2000) Miniaturization of HTS assays. *Innov. Pharmaceut. Technol.* 5, 12–17

11 Major, J. (1998) Challenges and opportunities in high throughput screening: implications for new technologies. *J. Biomol. Screen.* 3, 13–17

12 Johnston, P. (1998) Integration of screen development with HTS operations generates significant improvement in the lead generation process. *J. Biomol. Screen.* 3, 267–269

13 Burbaum, J.J. (1998) Miniaturization technologies in HTS: how fast, how small, how soon? *Drug Discov. Today* 3, 313–322

14 Parker *et al.* (2000) Development of high throughput screening assays using fluorescence polarization: nuclear receptor-ligand-binding and kinase/phosphatase assays. *J. Biomol. Screen.* 5, 77–88

15 Silverman, L. (1998) New assay technologies for high-throughput screening. *Curr. Opin. Chem. Biol.* 2, 397–403

16 Lightbody, B. and Alderman, E.M. (1999) Robotics development simplified. *Drug Discov. Dev.*, 50–52

17 Fox, S. *et al.* (1998) Seeking innovation in high-throughput screening. *Drug Discov. Dev.*, 32–37

18 Burbaum, J.J. (2000) The evolution of miniaturized well plates. *J. Biomol. Screen.* 5, 5–8

19 Ramsey, M. (1999) The burgeoning power of the shrinking laboratory. *Nat. Biotechnol.* 17, 1061–1062

20 Oldenburg, K.R. *et al.* (1998) Assay miniaturization for ultra-high

throughput screening of combinatorial and discrete compound libraries – A 9600-well (0.2 microliter) assay system. *J. Biomol. Screen.* 3, 55–62
21 Knapp, M.R. *et al.* (2000) Test tube's end. *J. Biomol. Screen.* 5, 9–12
22 Chiu, D.T. *et al.* (2000) Patterned deposition of cells and proteins onto surfaces by using three-dimensional microfluidic systems. *Proc. Natl. Acad. Sci. U. S. A.* 97, 2408–2413
23 Sundberg, S.A. (2000) High-throughput and ultra-high-throughput screening: solution- and cell-based approaches. *Curr. Opin. Biotechnol.* 11, 47–53.
24 You, A.J. *et al.* (1997) A miniaturized arrayed assay format for detecting small molecule-protein interactions in cells. *Chem. Biol.* 4, 969–975
25 Rose, D. (1999) Microdispensing technologies in drug discovery. *Drug Discov. Today* 4, 411–419
26 Vollert, H. *et al.* (2000) Wandel in der wirkstoffsuche – ultra-high-throughput-screening-systeme in der pharmaindustrie. *Transkript Laborwelt* 1, 5–10
27 Dunn, D.A. and Feygin, I. (2000) Challenges and solutions to ultra-high-throughput screening assay miniaturization: submicroliter fluid handling. *Drug Discov. Today* 5 (Suppl.), S84–S91
28 Brandt, D.W. (1997) Multiplex nanoliter transfers for high throughput drug screening using Biomek 2000 and the high desity replication tool. *J. Biomol. Screen.* 2, 111–116
29 Schaertl, S. *et al.* (2000) Performance testing of the EVOscreen® uHTS system. *SBS 6th Annual Conference and Exhibition, 6–9 September, 2000, Vancouver, Canada*
30 James, P. and Papen, R. (1998) Nanolitre dispensing – a new innovation in robotic liquid handling. *Drug Discov. Today* 3, 429–430
31 Lemmo, A.V. *et al.* (1998) Inkjet dispensing technology: applications in drug discovery. *Curr. Opin. Biotechnol.* 9, 615–617
32 Howitz, S. (1999) Components and systems for microliquid handling. In *Microsystem Technology: A Powerful Tool for Biomolecular Studies* (*BioMethods*, Vol. 10) (Köhler, J.M. *et al.*, ed.), Birkhaeuser
33 Berg, M. *et al.* (2001) Evaluation of liquid handling conditions in microplates. *J. Biomol. Screen.* 6, 47–56.
34 Moreau, M. *et al.* (2000) A micro-droplet dispenser based on a shock principle. *Actuator 2000, 19–21 June, 7th International Conference on New Actuators*, Bremen, Germany, B1.6
35 Gibbons, I. (2000) Microfluidic arrays for high-throughput submicroliter assays using capillary electrophoresis. *Drug Discov. Today* (Suppl.), S33–S37
36 Ramsey, M. (1999) The burgeoning power of the shrinking laboratory. *Nat. Biotechnol.* 17, 1061–1062
37 Pope, A.J. *et al.* (1999) Homogenous fluorescence readouts for miniaturized high-throughput screening: theory and practice. *Drug Discov. Today* 4, 350–362
38 Mere, L. *et al.* (1999) Miniaturized FRET assays and microfluidics: key components for ultra-high-throughput screening. *Drug Discov. Today* 4, 363–369
39 Mathis, G. (1999) HTRF technology *J. Biomol. Screen.* 4, 309–313
40 Hemmila, I. (1999) LANCE: homogeneous assay platform for HTS. *J. Biomol. Screen.* 4, 303–307
41 Sportsman, J.R. and Leytes, L.J. (2000) Miniaturization of homogeneous assays using fluorescence polarization. *Drug Discov. Today* (Suppl.), S27–S32
42 Auer, M. *et al.* (1998) Fluorescence correlation spectroscopy – lead discovery by miniaturised HTS. *Drug Discov. Today* 3, 457–465
43 Kask, P. *et al.* (1999) Fluorescence-intensity distribution analysis and its application in biomolecular detection technology. *Proc. Natl. Acad. Sci. U. S. A.* 96, 13756–13761
44 Kask, P. *et al.* (2000) Two-dimensional fluorescence intensity distribution analysis: theory and applications. *Biophys. J.* 78, 1703–1713
45 Moore, K. *et al.* (1999) Single molecule detection technologies in miniaturized high throughput screening: fluorescence correlation spectroscopy. *J. Biomol. Screen.* 4, 335–353
46 Haupts, U. *et al.* (2000) Macroscopic versus microscopic fluorescence techniques in (ultra)-high-throughput screening. *Drug Discov. Today* (HTS Suppl. 1), 3–9
47 Ramm, P. (1999) Imaging systems in assay technology. *Drug Discov. Today* 4, 401–410
48 Scheel, A. *et al.* (2001) Receptor-ligand interactions studied with homogeneous fluorescence-based assays suitable for miniaturized screening. *J. Biomol. Screen.* 6, 11–18
49 Turconi, S. *et al.* (2001) Developments in fluorescence lifetime-based analysis for ultra-HTS. *Drug Discov. Today* 6 (Suppl.), S27–S39
50 Eggeling, C. *et al.* (1998) Monitoring conformational dynamics of a single molecule by selective fluorescence spectroscopy. *Proc. Natl. Acad. Sci. U. S. A.* 95, 1556–1561
51 Lakowicz, J.R. *et al.* (1999) High throughput screening with multiphoton excitation. *J. Biomol. Screen.* 4, 355–361
52 Lightbody, B. and Alderman, E.M. (1999) Robotics development simplified. *Drug Discov. Dev.* 50–52
53 Karet, G. (2000) Factory automation speeds screening. *Drug Discov. Dev.* 34–40
54 Gentsch, J. and Bruttger, O. (2000) High throughput screening at the nanoscale. *JALA* 5, 60–65
55 Turner, R. *et al.* (2001) Screening in the nanoworld: single-molecule spectroscopy and miniaturized HTS. In *Handbook of Drug Screening* (Seethala, R. and Fernandes, P., eds.) pp. 589–608, Marcel Dekker
56 Mere, L. *et al.* (1999) Miniaturized FRET assays and microfluidics: key components for ultra-high-throughput screening. *Drug Discov. Today* 4, 363–369
57 Dunn, D. *et al.* (2000) Ultra-high throughput screen of two-million-member combinatorial compound collection in a miniaturized, 1536-well assay format. *J. Biomol. Screen.* 5, 177–187
58 Beggs, M. *et al.* (1999) The high throughput screening infrastructure: the right tools for the task. *J. Biomol. Screen.* 4, 143–149
59 Ladd, B. and Kenner, S. (2000) Information visualisation and analytical data mining in pharmaceutical RandD. *Curr. Opin. Drug Disc. Dev.* 3, 280–291
60 Ladd, B. (2000) Intuitive data analysis: the next generation. *Mod. Drug Disc.* January/February, 46–52
61 Hrib, N. (2000) Chemoinformatics: are we exploiting this new science? *Drug Discov. Today* 5, 483–485
62 Tropsha, A. (2000) Recent trends in computer-aided drug discovery. *Curr. Opin. Drug Disc. Dev.* 3, 310–313
63 Roberts, B.A. (2000) Screening informatics: adding value with meta-data structures and visualization tools. *Drug Discov. Today* (HTS Suppl. 1), 10–14
64 Zhang, J.H. *et al.* (1999) A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J. Biomol. Screen.* 4, 67–73
65 Zhang, J.H. *et al.* (2000) Confirmation of primary active substances from high throughput screening of chemical and biological populations: a statistical approach and practical considerations. *J. Comb. Chem.* 2, 258–265
66 Enal, S. *et al.* (2000) High-throughput genomics. *Mod. Drug Disc.* May, 41–42
67 Walters, P. *et al.* (1998) Virtual screening-an overview. *Drug Discov. Today* 3, 160–178
68 Cronin, M.T.D. (2000) Computational methods for the prediction of drug toxicity. *Curr. Opin. Drug Disc. Dev.* 3, 292–297