Miniaturisation of HTS assays

For high-throughput screening, recent studies indicate that the 1536-well plate density maintains the best balance between low volume, high throughput and data quality.

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Recent estimates are that it now takes approximately 15 years and between $300 and $600 million to develop the typical small molecule drug, and seven out of ten new drugs fail to recapture their R&D costs (1).

Motivations for miniaturisation

The pharmaceutical industry is experiencing increasing pressure to discover and develop innovative medicines, rather than the “me-too” and successive-generation drugs that have previously been the mainstay of the industry. This is not an easy task. The raw data - that is, therapeutic targets and novel compounds - are available from advances in functional genomics, combinatorial chemistry and high-throughput screening (HTS). The difficulty is not just finding the needle in the haystack, but finding the right needle in the right haystack. In order to do this, researchers need efficient and cost-effective ways to test more compounds against more targets, and sift through thousands upon thousands of data points to keep experimental efforts focused on only the best possible candidates for further development.

Historically, screening for compounds with activity against a specific target was done manually with a throughput of approximately 1,000 assays per day. The introduction of semi-automated screening methods increased that throughput five-fold, and fully automated screening methods increased the capacity to about 10,000 assays per day. Today, HTS methods allow pharmaceutical companies to screen 100,000+ assays per day.

With the available targets expected to increase by a factor of five or better, and compound libraries likely to grow even faster, current screening capabilities will not be enough to ensure success in the new millennium. Pharmaceutical companies are facing the daunting prospect of further expanding their screening capacities to remain competitive.

In deciding whether to adopt new screening technologies, most drug discovery companies are guided by two objectives: to increase throughput and decrease unit cost. Miniaturisation is one way to obtain both objectives. Typical assay volumes in 96-well plates are of the order of 100 microlitres, and the throughput potential is approximately 20,000 assays per day. With a 384-well plate, assay volumes can be reduced to 10 microlitres and throughput can reach 50,000 assays per day. Even higher-throughput, lower-volume formats are under development, including the 1536-well format and the laboratory chip.

Current trends

There are two emerging trends in the introduction of miniaturised formats to the HTS laboratory: evolutionary and revolutionary (2). The revolutionary approach involves moving directly from a 96-well plate to free format assays - that is, lab-on-a-chip and microscale total analysis systems. Laboratory chips are highly miniaturised, microfluidic reaction vessels printed or etched onto glass, plastic or silicon surfaces in the same fashion as are integrated circuits. These systems promise reaction volumes in the picolitre range and

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throughputs that will easily exceed 100,000 assays per day. A further discussion of this technology is beyond the scope of this article, but several good reviews are available (3, 4).

The evolutionary approach involves a gradual change from the 96-well plate to a 384-well plate and then higher densities. This approach is more feasible for pharmaceutical R&D, since instrumentation for higher density formats (that is, 384- and 1536-well plates) is becoming available - namely, scintillation counting and luciferase-based cellular reporter assays (2). The advantages of higher density formats versus the 96-well plate include more efficient storage, reduced cost due to smaller reaction volume and fewer disposables.

High density formats

The 1536-well plate can reduce reaction volumes to five microlitres or less, and provide throughput capacities of 100,000+ assays per day. The 1536-well plate is a four-by-four grid of wells within the same area as one well of the 96-well plate. Two versions of this format are available commercially: a deep-well (20 microlitres/well) plate from Greiner (Frickenhausen, Germany) and a more conventionally-shaped (two microlitres/well) plate from Corning (Acton, MA). The Corning plate, or wafer plate, was co-designed with Pharmocopia Inc (Princeton, NJ) and contains additional control wells and alignment features that enable and enhance its use with high-speed, high-precision instrumentation.

In the 1536-well format, shallow wells are evenly spaced at one-quarter the spacing of the 96-well plate; this allows pre-existing compound arrays in 96-well plates to be easily reformatted to 1536-well plates (5). The surface-area-to-volume ratio changes by only three- to four-fold as compared with the 96-well plate. Although evaporation was an initial concern, research has shown that this can be effectively managed through humidity control and ensuring that the plates are covered whenever possible. Many biological assays are amenable to the miniaturised 1536-well format, including cell-based assays. Research has shown that it is possible to grow cells in 1536-well plates and that, at confluence, a single well can support as many as a few thousand cells, which is sufficient for statistical significance (5).

Several miniaturisation strategies that allow densities higher than 1536 have been explored. "Field format" assays have been investigated at Pharmacopoeia and elsewhere (6); these utilise beads containing photochemically-releasable library compounds distributed in an immobilising matrix containing reagents to detect the presence of active inhibitors. Such assays allow for extremely high throughput but are limited to relatively soluble compounds that act independently.

Alternative container formats for bead-based assays have also been described. A 9600-well plate (0.2 microlitre volume) has been moulded from special, low-fluorescence plastic (7). The pyramid-shaped wells of this plate, together with the pointed intra-well boundaries, allow individual beads to segregate into defined wells. These wells then provide a container for subsequent biological evaluation. Evaporation at this volume becomes more significant but successful assays have been performed in this format. Finally, a 3456-well plate (one microlitre volume) has been disclosed by Aurora Biosciences (8). This plate is said to control evaporation through a novel, inverted-well design but it is not yet available commercially.

Technology for miniaturisation

Miniaturisation sounds like the proverbial "magic bullet"; however, the need for miniaturisation must be balanced against the scale. For miniaturised HTS to become a standard in the pharmaceutical industry, current assays must be adapted to run in smaller volumes, which might be impractical for certain assays. For example, it would be difficult to miniaturise radioactive binding assays that evaluate compounds as receptor antagonists. This is largely due to limitations of available detection hardware. Not surprisingly, then, it will be necessary for related technologies to advance in order to provide a complete engineering solution. The development of a massively parallel detection system for radioactivity could make the miniaturisation of radioactive assays more practical. Similar technological advances are required for reformattting, dispensing and detection before the full potential of miniaturisation of HTS will be realised.

Reformatting

One distinct problem with miniaturisation is reformatting. During any reformatting process, it is crucial that the sample stocks be handled and transferred without contamination. This requirement usually imposes a washable, or disposable, sample-handling system running in parallel. A recent evaluation by researchers at Glaxo Wellcome (5) indicated that most miniaturised formats (that is, 384-, 864- and 1536-well plates) could be read by commercially available readers after slight modifications, but the reformatting process often limits throughput.

Liquid handling

When dispensing reagents into miniaturised well plate formats, it is crucial to maintain a consistent volume of solution from well to well, and avoid cross-contamination. Inkjet dispensing technology, along with syringe-driven positive displacement, has recently been introduced into pharmaceutical R&D to meet these requirements (9). There are two main types of inkjet dispenser: piezoelectric- and solenoid-based systems. A piezoelectric-based system
Figure 1. Pharmacopeia has developed and implemented a variety of tools which enable microvolume biological assays in 1536-well plates. These include BlueBiRD™ (Figure 1a), a micro-drop liquid dispenser. BlueBiRD™ delivers liquid reagents via an eight-channel dispense head (1b) down to volumes of 50 nL per well, and can fill plates at a rate of 90 plates/hour. Charge coupled device (CCD) based field imagers (1c) can measure fluorescence and luminescence from entire plates within seconds. Highly accurate and precise volumetric liquid handling and optical detection are feasible with this instrumentation (1d), enabling robust screening assays, with narrow coefficients of variation.
Assay types which require the use of protein bound to plates or adherent cells require a means of transferring test compounds from production plates to test plates. Also, with these assay types, a means of washing reagents and materials from the test wells is required. Currently the only available instrumentation which supports washing of 1536 plates is the EMBLA-1536 marketed by Molecular Devices. There is no product currently available which supports plate-to-plate transfer at the 1536 level, although devices are being developed at Pharmacopeia.

**Detection methods** The advent of miniaturisation has accelerated the movement from radioactive to non-radioactive screening assays. Radioactive assays are an important tool in pharmaceutical research. In miniaturised formats, the use of radioactive compounds is impractical since the most common detection methods (serial scintillation) would require too much time (11). Non-radioactive strategies (that is, fluorescence or chemiluminescence) offer improved sensitivities with small sample volumes and decreased reagent costs. Most radioactive assays have an analogous fluorescence approach; however, conventional plate readers typically analyse one well at a time limiting the throughput of miniaturised HTS assays. A more practical approach uses charge coupled device (CCD) imaging to collect fluorescence or luminescence from all the wells at once. Although CCD imaging has shown promise in specialised HTS applications (11), the specific application of this technology for miniaturised assays is still in development. Work still needs to be done to find practical ways to isolate signal from individual wells, limit shading artifacts and background, and enable more advanced assay formats, such as fluorescence polarisation and time-resolved fluorescence. In addition, robust labeling reagents that fluoresce at longer wavelengths and offer enough versatility to allow for many kinds of fluorescence spectroscopy will need to be developed. Strategies such as fluorescence-correlation spectroscopy, time-resolved fluorescence, ratiometric dyes and fluorescence polarisation show promise as improved non-radioactive sample detection methods (12). Finally, both fluorometric enzyme- and cell-based luminescence assays have been shown to be amenable to miniaturised formats (11).

**Information management** Successful information management will be key to increasing productivity in pharmaceutical R&D (13). Historically, data were stored on paper; however, paper has limited accessibility and is difficult to update. Today, it is more common for information to be stored in data warehouses. The size of a data warehouse can be substantial - 1.2 Tbytes - and it is critical that information is aggregated and standardised so that researchers can effectively access and retrieve it to support decision-making (14). Informatics can provide a way to integrate data from disciplines such as functional genomics, combinatorial chemistry and HTS with data from more traditional sources such as medicinal chemistry, preclinical and toxicology testing, as well as literature and patent databases (15, 16). In this way, researchers can gain the capacity to answer increasingly difficult questions and make more complex decisions earlier in the R&D process.

A paradigm now exists to use informatics early in the discovery phase to "pre-screen" compounds and select only those that display good "drug-like" characteristics - that is, "forcing early fail" - helping companies concentrate efforts on the best possible choices early in the decision-making process (16). As the amount of data available for drug discovery continues to increase, informatics technology will play a larger and larger role in the success of R&D processes.

**Current status** Technology development for miniaturisation of HTS is highly dynamic and competitive. Although a significant amount of progress has been made with the 384-format, recent studies have indicated that the 1536-well plate density maintains the best balance between low volume, high throughput and data quality. Several companies are actively involved in the development of technology for 1536-well plates including: Amersham Pharmacia Biotech, CCS Packard/Packard Biosignal, Cartesian Technologies, JENOPTIC Bioinstruments GmbH, LJI Biosystems, Molecular Devices, Robbins Scientific, EVOTEC BioSystems, and PerkinElmer Wallac.

Currently, instrumentation is available for compound reformatting, simultaneous reading of plates, automated liquid handling and nanolitre dispensing. In addition, assays have been successfully "miniaturised" for 1536-format HTS, including fluorogenic enzyme, chemiluminescence, radioactivity, microparticle and cell-based assays.

**Conclusion** The scientific and technological advances outlined in this article represent significant progress in the miniaturisation of HTS; however, many challenges still remain, particularly in liquid handling (plate-washing, plate-to-plate transfer). A great need exists for miniaturisation of HTS for drug discovery and development, and a significant
amount of effort is focused on advancing science and technology for this purpose. It is very likely in the near future that the 1536-well plate system will replace the 96-well plate system as the standard in pharmaceutical R&D.

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References


