

Innovative chemical compound microarrays for drug screening

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As chemical libraries increase in size and, in the post genomic era, the number of possible drug targets grows, more advanced high throughput screening (HTS) technologies are needed. Miniaturisation of biological assays from 384-well plate to 1536- or higher well-formats has become the trend in today's assay development and lab automation processes in order to meet this demand; however, further increase in throughput is a challenge. Chemical compound microarrays, with the potential of screening millions of compounds per day in parallel against multi-targets, has given new insight into today's miniaturisation and ultra-HTS.

High throughput screening with microtitre plates, the major tool used for decades in the drug discovery world, faces a daunting challenge as a result of the fast growing numbers of drug targets arising from genomic and proteomic research, and the large chemical libraries being generated from high throughput synthesis. There is an urgent need to find new ways to profile the activity of large numbers of chemicals against hundreds of biological targets in a rapid and low-cost way. Miniaturising the reaction is one practical approach to increasing throughput without increasing the volume of assay reagents and the operation time. Therefore in the past decade most HTS screening has moved from a 96-well microtitre plate format to a 384-well system. In recent years, screening centres in several pharmaceutical companies as well as the USA National Institute of Health have also adopted the 1536-well plate format for many biochemical and cell based screening assays. However, the cost associated with implementing these systems is very high. For example, highly precise automated liquid handling is needed for delivering nanolitre to low

microlitre range volumes of reagents; a high resolution and sensitive plate detector is required for assay detection and fast data acquisition is necessary. In addition, assay development is more complicated, especially for cell-based assays, in view of the problems accompanying miniaturisation, such as evaporation when reactions are performed using these high density plates.

Chemical compound microarrays originating from genomic and proteomic research have given new alternatives for miniaturisation and assay automation for routine HTS. During the past few years, screening of chemical libraries on microarrays with different surface chemistries and activation strategies has generated many success stories [1-8]. The reagent costs when running these

microarray-based reactions are very low compared to well-based assays [Table1]. The requirements in order to establish a lab for running chemical compound microarray-based assays are relatively simple; for example, a microarray spotter and a chip scanner used for DNA and protein microarrays are good enough for arraying chemical compounds on to chips with different surfaces. For routine detection using immunoassays, a DNA microarray hybridisation chamber, washer and drier can also be used. In this brief review, we introduce a few different chemical microarray formats and their applications.

Small molecule microarrays (SMMs)

SMM is a chemical compound microarray format that covalently immobilises organic molecules on the surface of a slide. This was the first chemical compound microarray which emerged from DNA microarray technology in the late 1990s, and was greatly advanced by the different immobilisation techniques described by Dr Schreiber's lab at Harvard University [1]. The essential requirements for this form of chemical microarray are specific reactive groups on both the microarray surface and the compounds in the library. For example, compounds containing a thio-group are immobilised on the surface of

Format	Assay volume	Reagent Savings	Bio-reagent cost per reaction	Cost Savings
96-well plate	100-200 μ l	-	\$1.0 to \$2.0	-
384-well plate	20-50 μ l	2 to 10 fold	\$0.20 to \$0.50	2 to 10 fold
1536-well plate	2-10 μ l	10 to 100 fold	\$0.05 to \$0.10	10 to 40 fold
microarray	1-10 nl	> 1,000 fold	\$0.001 to \$0.005	>200 fold

Table 1. Reagent saving comparison of assays using plates and microarrays.

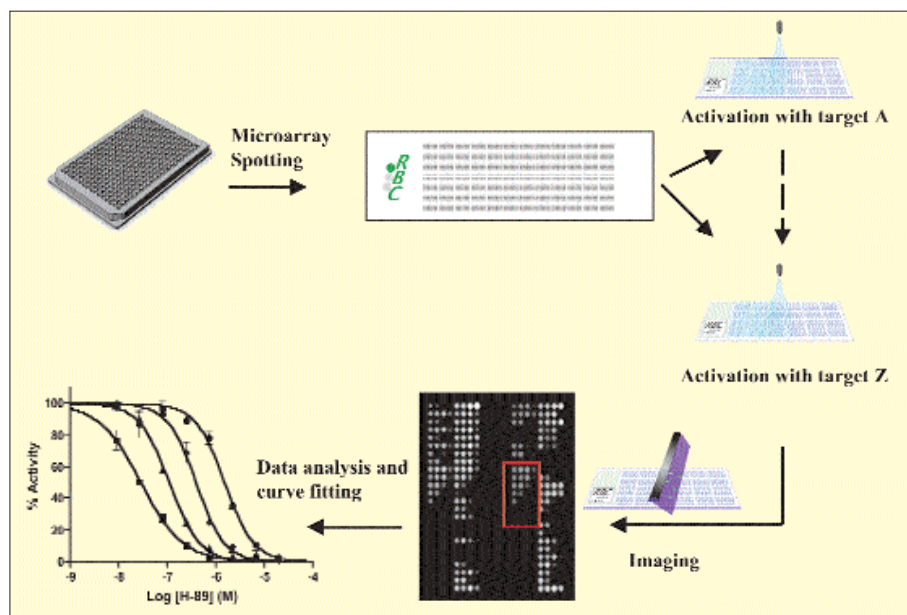


Figure 1. The process of the DiscoveryDot platform. Chemical compounds from an existing library in assay buffer containing DMSO and 10% glycerol are arrayed on multi-sets of slides including substrate-coated slides for heterogeneous assays, which are then activated with different drug targets by aerosol deposition. After incubation, the slides are processed accordingly and imaged by a laser scanner. The information on fluorescence intensity obtained is then analysed for lead identification in HTS or curve fitting for IC₅₀ in profiles.

maleimide-derivatised glass, an alcohol reactive glass surface is used for immobilising compounds containing hydroxyl functionalities, covalently linked small molecules with peptide nucleic acid tags are arrayed on an oligonucleotide surface, azide-functionalised molecules are arrayed on a phosphane-derivatised surface, and various photochemistries allow cross-linking of chemicals to microarray surfaces.

SMMs are particularly useful for probing binding partners or identifying targets; Kuruvilla and coworkers have microarrayed and screened 3,780 newly synthesised structurally complex 1,3-dioxane small molecules on glass slides with a fluorescently (Cy5)-labelled yeast prion-like protein Ure2p, a repressor of transcription factors for Nil1p and Gln3. One out of eight hits from the primary binding screening had activity in a secondary cell-based screening assay with a dissociation constant (K_D) value of 18.1 μ M [1]. However, the immobilisation step of the SMM format has limited use with exist-

ing chemical libraries. In addition, factors such as the length and flexibility of linkers, compound binding orientation, spatial hindrance, and microarray surface properties also affect target binding.

Micro arrayed compound screening (μ ARCS)

MicroARCS is a format of chemical compound microarray that arrays and dries organic molecules dissolved in DMSO on polystyrene sheets. This approach has been developed by scientists in Abbott Laboratories [2], and licensed by Discovery Partners International for commercialisation. The advantage of this format is that ARCS can deposit any chemical compound library in a dry form on to a polystyrene sheet, and further biochemical assays can be preformed in an aqueous rich environment. Scientists have created and laid agarose gels containing biological targets on top of the compound sheet, allowing the compounds to dissolve and diffuse into the gel and interact with the target. The bio-

logical reaction is then initiated by placing a second gel containing substrate on top of the target gel. Many targets have been tested with the ARCS platform since its inception, including enzymes and receptors. However, one of the major problems with this approach is that the rate of resolubilisation and diffusion of different classes of dry compounds complicates the dynamic range of arrayed compounds versus bio-available compounds.

DiscoveryDot: solution-phase chemical microarrays

In order to create chemical compound microarrays that can perform traditional homogeneous biochemical reactions and avoid the drawbacks mentioned above in the SMM and ARCS formats, Dr Diamond's group from the University of Pennsylvania created a solution-phase chemical microarray, using glycerol as an anti-evaporating reagent mixed with chemical compounds which were arrayed on the microarray surface. An aerosol deposition technology was used to activate the microarray, converting biological targets into a fine mist, which could be overlaid on top of the whole microarray simultaneously [3]. To exploit this technology as a new tool for HTS, Reaction Biology Corporation has licensed and developed it into a commercial product, the DiscoveryDot platform [4-7]. The technology microarrays 6,600 organic compounds on a standard 1x3 inch glass slide in a non-volatile glycerol-based format compatible with -80°C storage. Each compound forms an individual well-less reaction centre in a volume of one nanolitre. Since the microarrayer easily microarrays many slides at a time, these replicated slides can be screened in parallel against multiple targets [Figure 1]. The chemical compounds and targets are in solution throughout the process, therefore any existing chemical libraries can be screened by this platform, making the technology the only universal chemical

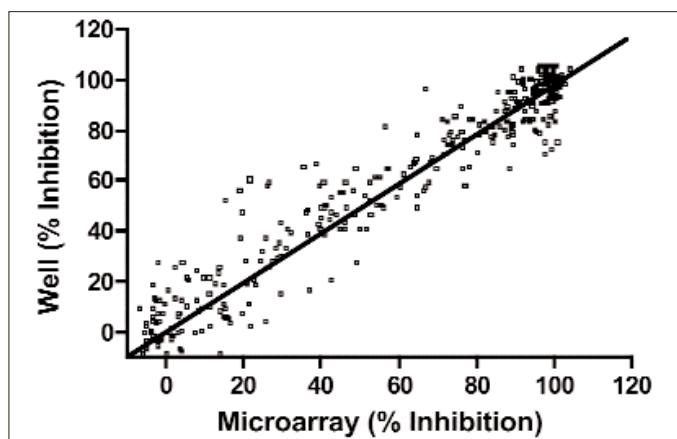


Figure 2. Correlation of screening data obtained by DiscoveryDot versus well-reaction. A small focused chemical compound library was screened against Src tyrosine kinase to compare the microarray reaction with the well-based reaction. The well-based reaction was performed using a ProFluor Src-family kinase assay kit following Promega's protocol, and the microarray reaction was performed with ELISA-based detection using peptide substrate-coated slides. The data were expressed as % inhibition. The overall correlation, R^2 , was 0.918.

microarray to date. The key component of the DiscoveryDot utilised is the aerosol-deposition technology, which can activate thousands of chemical compounds on microarrays with microlitre volumes of biotargets. Taking advantage of miniaturisation pays off in larger scale HTS, where the total cost of microarray-based reactions could be significantly less than that incurred with standard low-volume 384-well based assays [Table 1]. A standard genomics or proteomics lab can run this assay by acquiring an aerosol activator from Reaction Biology Corporation. DiscoveryDot has been used for both primary/secondary screening and for target profiles against enzymes like kinases, phosphatases, and proteases with the purified enzyme or the cell lysates [3-7]. Since the platform mimics conventional well-based systems, most well-based homogeneous assays are transferable to the platform, such as the Vivid P450 assay (Invitrogen), the ProFluor kinase assay (Promega) and the Z'-Lyte kinase assay (Invitrogen). In addition, heterogeneous assays such as radioisotope- or ELISA-based assays can also be performed on the DiscoveryDot platform, which eases the problematical multi-step washing necessary in well-based reactions [5]. The screening data obtained from the microarray had good correlation with data obtained from well-based reactions [Figure 2]. However, the present limitation of the DiscoveryDot system is that microarray detectors currently available cannot detect assay formats such as fluorescent polarisation and time-resolved fluorescence energy transfer, and plate imagers which have these capabilities cannot perform at the high resolution needed for microarray detection.

Chemical compound microarray for cell-based screening

Recently chemical compound microarrays have entered a new territory; cell based high content screening (HCS). By using a biodegradable poly-(D),(L)-lactide glycolide (PLGA) copolymer to cage organic molecules, Dr Sabatini from Massachusetts Institute of Technology and Dr Stockwell from Columbia University have impregnated a microarray surface with 200 μ m diameter discs for each compound to be screened. Cells were then seeded on top of these compounds that were allowed slowly to diffuse out to affect the proximal cells [8]. PLGA, which has been used to deliver protein drugs, enabled the controlled release of proteins and small molecules through a combination of drug diffusion and polymer erosion. This chemical microarray technology has the potential to be used for high throughput cell based assays, but a few obstacles must be overcome before it can become useful as a broad platform. For example, finding one polymer for imbedding and releasing possibly millions of chemical compounds with different physical properties is quite a challenge. In addition, the release as well as the stability of chemicals will affect the assays as well as comparison studies.

Conclusion

The new development of chemical compound microarray technology, capable of analysing millions of chemicals with multiple biological targets in parallel, will further advance HTS via its miniaturised reactions and parallel screening system. Its application in target identification, hit identification and lead optimisation have made it a promising platform, useful in many drug discovery processes to reduce costs and shorten cycle times while increasing productivity. The solution-phase chemical microarray, which mimics the conventional well-based system, can be used for both homogeneous and heterogeneous assays with virtually any chemical compounds, enzyme reactions, protein-protein/peptide interactions, and possibly receptor-ligand binding. However, in order to take full advantage of chemical compound microarrays, further improvements in the precision of the microarray, as well as the development of multi-functional detectors and multi-task data analysis software are necessary. To achieve this, the potential and scope of chemical compound microarray technology must be more widely recognised.

References

1. Kuruvilla FG *et al.* Dissecting glucose signalling with diversity-oriented synthesis and small-molecule microarrays. *Nature* 2002; 416: 653-657.
2. David CA *et al.* Microarray compound screening (microARCS) to identify inhibitors of HIV integrase. *J Biomol Screen* 2002; 7: 259-266.
3. Gosalia DN and Diamond SL. Priting Chemical libraries on microarrays for fluid phase nanoliter reactions. *Proc Natl Acad Sci USA* 2003; 100: 8721-8726.
4. Ma H, Horiuchi KY, Wang Y, Kucharewicz SA, Diamond SL. Nanoliter Homogeneous Ultra-High Throughput Screening Microarray for Lead Discoveries and IC50 profiling. *Assay Drug Dev Technol* 2005; 3: 177-187.
5. Horiuchi KY, Wang Y, Diamond SL and Ma H. Microarrays for the functional analysis of the chemical-kinase interactome. *J of Biomol Screen* 2006; 11: 48-56.
6. Horiuchi KY, Wang Y and Ma H. Biochemical microarrays for studying chemical biology interaction: DiscoveryDotTechnology. *Chemical Biology & Drug Design* 2006; 6: 87-88.
7. Ma H, Wang W, Pomaybo AS and Tsai C. A Homogenous Microarray for Enzymatic Functional Assays. *Frontiers of Biochip Technology* 2006; 3-18. Springer, New York.
8. Bailey SN *et al.* Microarrays of small molecules embedded in biodegradable polymers for use in mammalian cell-based screens. *Proc Natl Acad Sci U S A* 2004; 101: 16144-16149.

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