High-throughput screening

Liquid-handling systems for 384-well applications

There is a consensus of opinion in the industry that miniaturization of high-throughput screening (HTS) is cost-effective and that it significantly increases screen capacities and throughput. The drive towards 384-well assays for HTS, and further miniaturization beyond the 384-well format, requires precise, low-volume liquid-dispensing instrumentation. Such devices also permit the direct addition of compounds from a liquid stock plate to an assay plate, simplifying the compound supply process for HTS.

Several liquid-handling systems are available, some of which were represented at the recent conference on Microplate Technologies (MiPTec '97) held on 23–25 June in Arlington, VA, USA. Currently available liquid-handling systems for HTS fall into three categories:

- robotic sample processors (RSPs),
- automated pipetting devices and
- bulk reagent delivery systems.

Robotic sample processors

RSPs are used in most HTS programmes and compound supply facilities, and have been integrated into large robotic HTS systems. The Tecan Genesis (Tecan, Hombrechtikon, Switerland) and the Hamilton Microlab 2200 (Hamilton, Reno, NV, USA) are versatile systems with a multichannel (usually 8) dispensing head that can move in an XYZ axis and address both 96- and 384-well plate formats. In particular, the Hamilton with its fine needles is capable of adequate liquid-handling procedures in 864-well plates. In the experience of the Glaxo Wellcome team, the volume limit with needles with either system is 0.5 µl, but nanolitre dispensing is possible using various gridding tools. Of the other equipment manufacturers, Beckman (Fullerton, CA, USA) have now released software (BioWorks) to enable dispensing into 384-well plates on the Biomek 2000.

Automated pipetting devices (96/384 channel dispensers)

The Matrix PlateMate (Matrix Technologies, Lowell, MA, USA) is manufactured by Opal Jena (Germany) and is increasingly being used for HTS. The PlateMate accommodates 96-, 192- and 384-well microtitre plate configurations and uses two piston sizes to cover a dispense/aspirate volume range of 1–250 µl. The PlateMate uses disposable tips, which can be easily changed manually or washed by the onboard wash station. The PlateMate is designed to perform microplate replication (96-to-96 or 384-to-384) and can translate 96-well plates into 384. In addition, the PlateMate can add bulk reagents across an entire plate using its reagent reservoir. The PlateMate has an RS232 serial port and can be supplied configured with single or double plate stackers and a stepper motor-controlled X–Y table with 0.1 mm resolution. The PlateMate system's PC-based Windows application software allows custom protocol creation.

The Robbins Hydra-96 liquid microdispensor (Robbins Scientific, Sunnyvale, CA, USA) has also been widely used in genomics and HTS laboratories, but until recently only two versions of the instrument were available: one with 290 µl and a second with 580 µl syringes. Robbins reported at MipTec and in their own technology update magazine [*Innovations* (1997) Spring edition, 5(2)] that they have six new models of the Hydra available including their latest innovation, a 384-well syringe version. The Hydra-384 is fitted with 100 µl syringes arrayed in 4.5 mm spacing and each syringe has a teflon coated 26 gauge needle with a 0.017 inch external diameter. The Hydra-384 is capable of delivering volumes as low as 100 nl with high precision. Robbins have developed the Hydra-384 specifically to meet the needs of HTS laboratories standardizing on the 384-well plate as a screening format. Interestingly, another version of the Hydra-96 – the Hydra-R/B – has been developed to dispense beads used in scintillation proximity assays and resins for combinatorial chemistry without clogging. This instrument is available with either 290 µl, 580 µl or 1 ml syringes. All of the Hydra instruments have an RS232 port for communication links, and the Hydra-96 has been integrated into HTS robots. Robbins have also just launched a web site which has an HTS section (http://www.robsci.com). A robotic version of the Hydra is available in one of two options. The first has an X–Y microscope stage (two position) for automated loading and positioning of plates on any Hydra with an accuracy of 5 µm. This is intended for use with high-density formats beyond 384-well plates. The second option is a small, three-axis robotic workstation, with two stackers that have a capacity for 40 plates.

Tomtec (Hamden, CT, USA) have also been supplying liquid-handling devices for HTS for some time. At MipTec, Tomtec introduced a new expanded range of the Quadra model 220 series. One of the new models is the Quadra 220-384 for 384-well pipetting, which has a two-position X–Y shuttle for 384-well plates. The head of the machine has 384 stainless steel tips (external diameter approximately 0.5 mm) and uses positive displacement for liquid delivery. The tip height is programmable and the volume range of the machine is 1–50 µl. The Quadra is configured for robotics integration and has a RS232 serial interface.

Zymark (Hopkinton, MA, USA) mentioned at MipTech that they are developing a 384-well version of the AutoPlate96 (RapidPlate96), which should become available soon.

Bulk reagent delivery systems

Labsystems (Helsinki, Finland) have developed a 96/384-well Multidrop. The Multidrop utilizes a high-precision peristaltic pump to dispense from single or multiple reagent bottles. The Multidrop can handle both 96- and 384-well formats with a volume range of 20–395 µl in 5 µl increments (for 96-well plates) and 10–100 µl in 5 µl increments (for 384-well plates). This machine can

rapidly fill a 96-well plate with 20 µl per well in 5 s and a 384-well plate with 20 µl per well in 14 s with good precision. The plate moves under the 8-channel dispensing head and in the 384-well mode, each plate is filled by two passes under the head. The plate is offset on the second pass to allow alternate rows to be filled. The 96-well version of the Multidrop has been widely used for dispensing cells and has been integrated onto many HTS robotic systems. The Multidrop has a serial RS232C interface for communication with PCs.

BioRobotics (Cambridge, UK) have developed a 96/384-well bulk dispensor called the BioFill. The BioFill has a 16-probe dispense head attached to a cross slide that allows access to four plates on the stage of the instrument. The BioFill can add a single reagent to an entire 384-well plate in 40 s. In 384-well mode, the BioFill has a volume range of 20–90 µl in 10 µl increments. During the filling procedure, the probes can optionally be lowered to the bottom of the well and withdrawn as the well fills thereby, as BioRobotics claims, minimizing bubbles or drops at the end of the probe.

Nanolitre dispensing

Some companies are developing very low volume dispensers but there are significant issues relating to the accuracy of dispensing low volumes in high density formats and speed of dispensing which need to be addressed before these systems can be used for HTS.

Piezoelectric technology. Drs Roeland Papen and Al Kolb from Packard Biosciences (Meriden, CT, USA) reported on the development of their MultiPROBE nl (a nanolitre liquid-handling workstation) and the BIOCHIP processor for liquid handling in miniaturized screening formats. The MultiPROBE has been 'developed with piezo-drop-on-demand technology to a high precision XYZ-robot with integration of aspiration and positive feedback'. This allows for accurate nl dispensing of reagents. Volumes from 250 pl to 25 µl can be dispensed, allowing direct dilution of compounds from stock solutions. It is also possible to set up dilution curves spanning five orders of magnitude directly from stock solutions of compounds. Packard's piezoelectric dispensing system is based on glass capillary tubes with a bonded ceramic collar. Voltage applied to the ceramic collar causes contraction that results in dispensing a 200 pl droplet. A high frequency voltage pulse causes multiple droplet release; a frequency of 1,000 Hz results in the dispensing of one 200 nl droplet per second. In addition, the system has built in ultrasonic cleaning of the capillary tubes. The Packard system will dispense compounds without predilution directly into miniaturized assay volumes. Data from a cell-based luciferase reporter gene assay substantiated these claims. It is envisaged that the MultiPROBE nl will be one of a new generation of nanolitre dispensing systems that will make miniaturization of HTS a realistic short-term goal.

Inkjet technology. A group from Cartesian Engineering (Durham, NC, USA) reported at MipTec on their nanolitre four-channel inkjet dispensing system for microscale HTS assays. Cartesian are developing solenoid-based inkjet dispensing to deliver chemical or biological reagents to high-density formats. Cartesian state that 'the technology couples the precision of a syringe pump with the high speed, nanolitre dispensing capabilities of a solenoid inkjet valve for nanolitre dispensing and aspiration capabilities'. Cartesian claim that their system has a large volume range (4 nl to 4 ml) and accurate dispense capabilities. In addition, Cartesian point out that because the dispense tips do not touch the liquid, no tip wash step is required and rapid dispense times are possible taking seconds per plate.

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Emerging molecular targets

Gelatinase A and platelet activation

Gelatinase A, also known as matrix metalloproteinase-2, MMP-2 and type IV collagenase, is important in the remodelling of the extracellular matrix. Now Grzegorz Sawicki and coworkers at the University of Alberta (Alberta, Canada) have presented data that suggest that gelatinase A may also play an important role in platelet aggregation.

The Canadian investigators found that small amounts of gelatinase A are released in a dose-dependent manner when platelets are activated by collagen or thrombin. A specific antibody against gelatinase A, as well as two different inhibitors of the enzyme, phenanthroline (Sigma) and SC44463 (Pfizer), inhibited the aggregation of platelets that normally occurs in response to either collagen or thrombin. Addition of recombinant gelatinase A to stirred platelets did not trigger aggregation, but it did amplify the aggregatory effects of collagen. Most intriguing, the investigators report that phenanthroline inhibited platelet aggregation that is insensitive to inhibition by aspirin and apyrase, which suggests that the mechanism by which gelatinase A inhibits platelet aggregation is independent of the activation pathway of thromboxane A_2 and ADP [*Nature* (1997) 386, 616–618].

The researchers believe that gelatinase A may prove to be an effective new target for the discovery of antiplatelet agents. It is released from the platelet as an inactive proenzyme of 72 kD and then processed into an active 64 kD form. Either gelatinase A itself or its processing enzyme is likely to serve as an effective target for drug discovery.

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