Continued miniaturisation of assay technologies drives market for NANOLITRE DISPENSING

The continued miniaturisation of assay technologies in high throughput screening, compound management and protein crystallisation has created a pressing need for improved nanolitre liquid handling systems. A recent industry market survey suggests that although the total liquid handing market in pharma drug discovery is contracting, the nanolitre dispensing segment is expected to grow to $56 million US dollars in 2004 with a 45% annual growth rate. Growth over the next two years is predicted to be especially strong for devices based on acoustic transducers and solenoid sensors. This review takes a look at some of the latest offerings in this increasingly competitive market place and discusses some recent industry trends. Customer expectations have now shifted from lower volumes to improved performance and reliability, added value and maximising the quality to price ratio.

By Dr John Comley

Two years ago in ***Drug Discovery World*** we reviewed nanolitre dispensing in pharma drug discovery and highlighted the need for instrumentation that could support enabling miniaturised assays (in volumes of <5µL)[1]. Specifically we identified nanolitre compound reformatting (direct nanolitre transfer of compounds into assay plates with no intermediate dilutions) and low volume bulk reagent addition to miniaturised assays as key liquid handling processes in need of improved robust liquid handling systems. Two years on we see an increasing diversity of products available or emerging to support these processes and protein crystallography (see **Table 1**), but are customers now convinced of the maturity of the technology, is now the time to invest more in nanolitre dispensing and to consider replacement of older, obsolete or first generation systems?

Survey points to growing market

If the results of a recent industry market survey (HTStec's Nanolitre Dispensing Trends 2004) are correct then we can expect to see significant growth in this segment of the liquid handling market over the next few years, while sales of more traditional dispensers and pipettors with a higher volumetric capacity are predicted to decline marginally in the pharma sector (see **Figure 1**). As a proportion of the total liquid handling budget the percentage spent on nanolitre dispensing is predicted to rise from 16% in 2003, to 27% in 2004, to 42% in 2005, representing annual growth rates of 45% in 2004 and 65% in 2005.

In **Table 1** we have listed some examples of the ever increasing variety of nanolitre dispensing devices that are available today or are in development. Instruments are split into dispenser groups mainly on the basis of their underlying dispensing

Table 1: Some of the increasing variety of low volume and nanolitre dispensing devices now available

* KEY to Main Use: A = well auditing, BD = bulk reagent dispensing, CR = compound reformatting, DD = direct dilution, M = microarraying and arrayplates, PC = protein crystallography, PR = module in production instruments

NANOLITRE DISPENSER GROUP	CHANNELS	MAIN USE*	INSTRUMENT EXAMPLES	WEBSITE
CONTACT DISPENSERS				
– Pin Tools	96 to 1536 pins	CR	V&P Scientific	www.vp-scientific.com
		CR	Beckman	www.beckman.com
		CR	CyBio	www.cybio-ag.com
		CR	PerkinElmer LAS	www.perkinelmer.com
– Positive Displacement Micropipettes	8 to 16 per strip	PC, CR	TTP Labtech – mosquito™	www.ttplabtech.com
PRESSURE BOTTLE				
– Solenoid Pressure Bottle	1 to 32	BD	PerkinElmer LAS – FlexDrop™	www.perkinelmer.com
		BD	Aurora Discovery – FRD™	www.auroradiscovery.com
		BD	Genetix – aliQuot	www.genetix.com
		BD	Molecular Devices – AquaMax™ DW4	www.moldev.com
– Membrane Valve Pressure Bottle	1 to 8	BD	CyBio – CyBi™-NanoJet	www.cybio-ag.com
VALVE SYRINGE				
– Solenoid Syringe	1 to 16	BD, PC	Genomic Solutions – synQUAD™, Honeybee™	www.genomicsolutions.com
		BD, PC	Gilson – Constellation™ 1200	www.gilson.com
		BD, PC	Innovadyne – Nanofill™, Nanodrop	www.innovadyne.com
– Solenoid Syringe	96	CR, PC	Innovadyne – Screenmaker™	www.innovadyne.com
– Smart MicroValve Tip	1 to 8	BD, PC	Deerac Fluidics – Equator™	www.deerac.com
SOLENOID SENSOR	96	CR, DD, M	CaliperLS – Sciclone inL10™	www.caliperls.com
CAPILLARY SIPPER	96 or 384	CR, PC	Genomic Solutions – Hummingbird™	www.genomicsolutions.com
INTEGRATED DISPENSE & STORAGE DEVICES	364 wells	CR	Boston Innovation – SmartPlate	www.bostoninnovation.com
	384 to 1536wells	CR	IMTEK DWP™ (Dispensing Well Plate)	www.imtek.de
ACOUSTIC TRANSDUCER	1 transducer	A, CR, DD	Labcyte® – Echo 550	www.labcyte.com
		A, CR, DD	EDC Biosystems – HTS-01	www.edcbiosystems.com
OTHER				
– Piezo	1 to 16	BD, CR	Evotec Technologies – Mona, Dina	www.evotec-technologies.com
		CR, M	Gesim – Nano-Plotters™, Dispensers	www.gesim.de
		M	Scienion – sciFLEXARRAYER	www.scienion.de
		M	PerkinElmer LAS – Piezorray™	www.perkinelmer.com
		CR, DD, M	Aurora Discovery – PicoRAPTR	www.auroradiscovery
– Piezo	96 or 384	CR	Aurora Discovery – MPD	www.auroradiscovery.com
– Ceramic Pump	8	BD	CyBio – CyBi™-Drop	www.cybio-ag.com
– Electro-Magnetic Bellows	1	PR	Fluilogic/OBPW – EMB Pump	www.obpw.com, www.fluilogic.fi

mechanism and the number of dispensing channels, although a few unrelated technologies are grouped into the 'other' category. Apart from a few contact devices (pin tools and positive displacement micropipettes) all the instruments listed are non-contact, and this illustrates the almost total acceptance of the fact that non-contact dispensing technologies are advantageous for low volumes and high density formats. Furthermore, the survey ranked non-contact dispensing and minimal or zero dead volume as the most desirable attributes in a nanolitre dispenser.

The breakdown of unit sales in 2003 and that predicted for 2005 by the survey for these same dispenser groupings is given in **Figures 2** and **3**. The findings suggest that devices within the dispenser group categories valve syringe and pressure bottle currently account for the greatest number of sales (up to 300 units per year) and will continue to dominate up to 2005. The majority of current valve syringe sales in 2003 were from protein crystallography groups, the only other instrument category with significant sales to protein crystallography groups are devices based on positive displacement pipettes (ie TTP Labtech mosquito™). The next best selling dispenser group categories (about 50% less unit sales than valve syringe and pressure bottle groups) are the capillary sipper and pin tools. Sales of both are predicted to be sustained at around 150 units in 2005. Despite the limitations of pin-tools, in terms of their performance, the survey has found significant supporters of their use, possibly due to frustration over the robustness of alternative non-contact instruments, the comparative low cost of pin tools and lack of alternative highly parallel (384 and 1536) nanolitre dispense heads. The only dispenser group categories that are predicted to show real growth in new instrument sales in 2004 and 2005 are devices based on a solenoid sensor, acoustic transducer and integrated dispense and storage devices. We will now consider some of the dispenser group categories highlighted in the survey in more detail and discuss their potential to add value to the dispensing process.

Capillary sipper proves market for parallel non-contact processing

The introduction of GSI's Hummingbird™ not only clearly demonstrated the latent demand in pharma for devices able to facilitate parallel non-contact nanolitre transfers, but directly contributed to enabling low volumes assays in 384 and 1536 plates in many companies. The product has undergone a number of design changes since its introduction several years ago, these were initially focused on making capillary replacement simpler and extending capillary access to deep well plates. Most recently the Hummingbird's capillary dispense heads were redesigned so that they are easily exchangeable for a variety of 'fixed volume dispenses'. Hummingbird cassettes are now available in 25, 50, 100, 250, 500 and 1,000nL volumes and are colour-coded so that a user can easily spot what volume they are working with. Other improvements include more user-friendly software

Figure 1: Predicted growth in sales of nanolitre dispensing devices

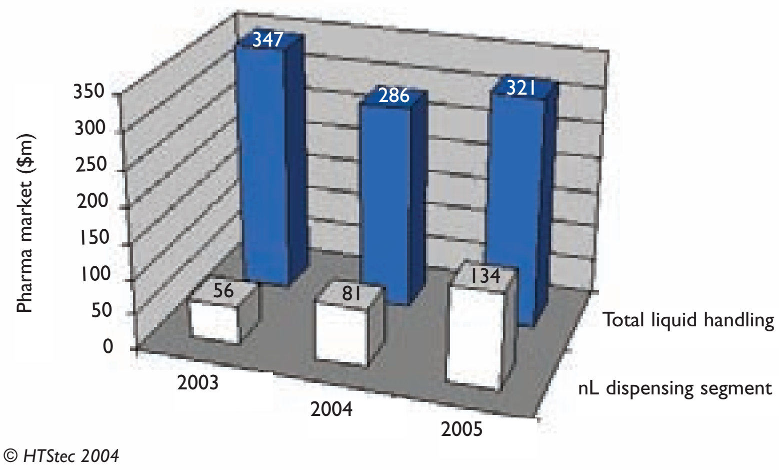


Figure 2: Breakdown of nanolitre dispensing unit sales (% of all units sold) in 2003

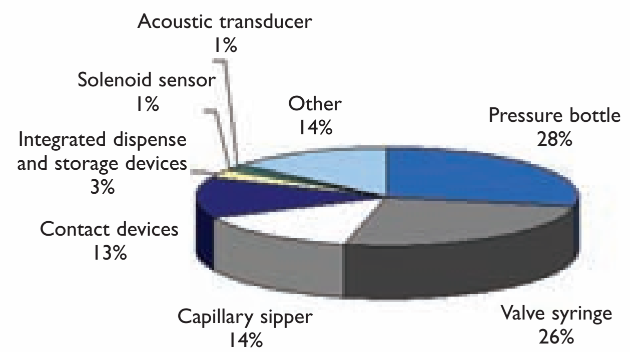
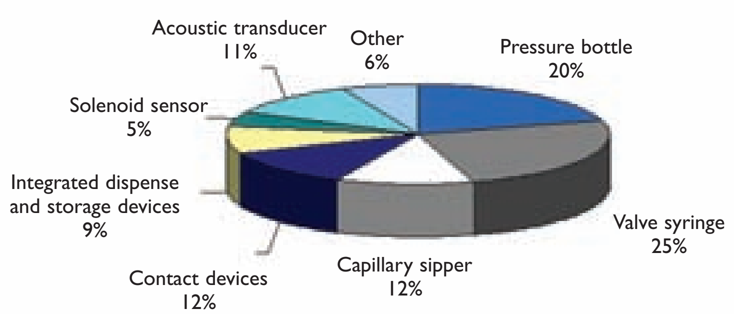
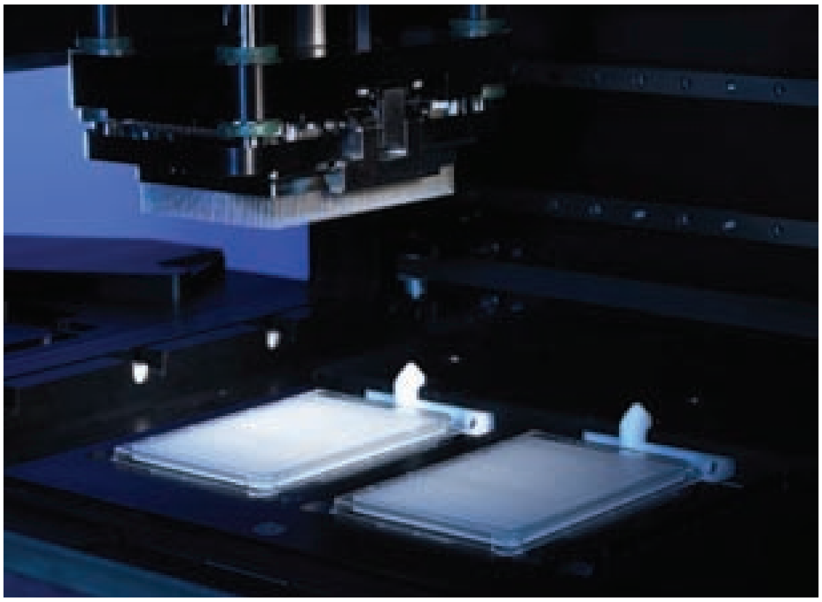


Figure 3: Predicted breakdown of nanolitre dispensing sales (% of all units sold) in 2005





Molecular Devices' FLIPR$^{TETRA}_{TM}$ 1536 parallel dispense head based on air displacement technology

and built-in plate sensors, so that if integrated within a production run a plate handler fails to place a plate, the system will shut down so that you don't waste valuable reagents.

However, credit must go Molecular Devices as the new FLIPR$^{TETRA}_{TM}$ is the first commercial product offered with a parallel 1536 dispense head capable of dispensing to all 1536 wells in one shot. The FLIPR is the most widely-used screening system for GPCR analysis and with FLIPR$^{TETRA}_{TM}$ researchers can now increase the throughput and reduce the cost of their calcium mobilisation and membrane potential assays even more, screening over 3,000 kinetic assays every six minutes using a few thousand cells per assay. This throughput is achieved using a novel liquid transfer device that simultaneously aspirates and then dispenses into all 1536 wells in parallel. The fluidics head is composed of 1536 individual piston-driven cannulas. Operation is by simple air displacement, with liquid only contacting stainless steel. The novel aspect of the device is a proprietary technology that replaces individual seal gaskets with a single elastomeric gasket for the entire array of tips. This contact-based dispensing system is designed for routine liquid transfers in the range of 0.5 to 3µL.

Genetix aliQuot bulk reagent dispenser with pressure bottle, single solenoid and multiway manifold

Easy accessible low-volume bulk reagent dispensing still wanted

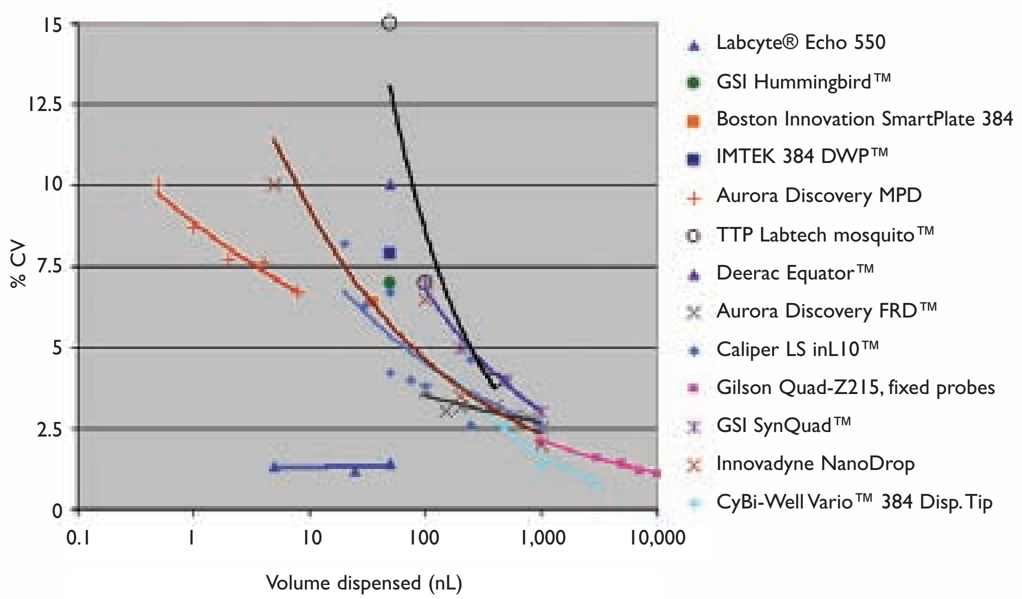
Between three to five years ago valve syringe devices represented the best and in some case the only way to add bulk reagents in low volumes to high density formats in a fast non-contact manner. However, the high price of these systems and their complexity, particularly with respect to their control software has restricted the wider use of these instruments beyond the small number of experienced end-users. What the typical customer interested in adding bulk reagents for miniaturised assay assembly really wants now is an instrument comparable in simplicity and cost to the Thermo Multidrop, but with a lower volume (down to 1.0 or possibly 0.5µL) and 1536 capability. One of the biggest challenges for the wider take up of 1536 is the amount of expertise and time scientists have to spend setting up, maintaining and optimising dispensers that have the necessary precision and accuracy. As **Table 1** lists there are lots of dispenser options today, but very few are easy to use and truly robust (ie hard to break, easy to fix and don't need to be retuned daily). Ideally the user should be able to use it after less than 15 minutes training and require no more than 2-3 minutes to set up the machine before starting dispensing. It is interesting to observe that few of the valve syringe devices with the capability to dispense down to 50nL are actually used today for bulk additions much below 1.0µL, and the aspirate function can, under some circumstances, be a hindrance for continuous bulk dispensing or bead-based applications. The importance of devices incorporating a pressure bottle (28% of all nanolitre dispenser purchases in 2003) highlights the desire for simpler bulk reagent filling and instruments like PerkinElmer LAS FlexDrop and Molecular Devices AquaMax™ have had a significant impact on the development of this market. However, the desire for instruments that are still cheaper, easy to operate and maintain, yet retain the ability to fill 1536 plates with 1µL at <10%CV in under a minute has now been addressed by Genetix's newly launched aliQuot. The aliQuot's pressurised bottle reservoir has a single valve with a common fluid path feeding a multi-way pressure manifold. A microcontroller maintains constant head pressure by continually monitoring and adjusting the pressure within the reservoir as the volume remaining changes. Precise design and fluid path modelling (using CFD) ensures uniform dispense volume across the manifold with minimal air bubble formation. The nice thing about the manifold is that it is easy to remove, disassemble, sonicate, autoclave, sterilise and clean. The aliQuot's lightweight, compact and portable design also means it fits inside flow cabinets for sterile dispensing. But above all the ailQuot's price to quality ratio (currently priced at just under £10,000 in the UK) makes 1536 bulk dispensing affordable and accessible, and facilitates the purchase of multiple or even standby units.

Labcyte® Echo 550 true non-contact dispenser based on an a acoustic transducer

Acoustic transducers bring added value

The greatest potential for future growth in nanolitre dispenser sales was predicted in the survey for devices based on an acoustic transducer, with sales in excess of 100 units estimated in 2005. The implementation of the first commercially available platforms based on an acoustic transducer in late 2003, supporting true non-contact dispensing (ie the ejection of liquid drops from a source without entry of a dispensing element into the source or contact with destination substrate) represented a

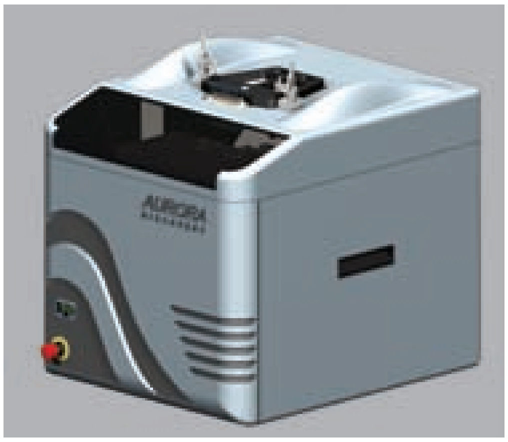
Figure 4: Effect of volume dispensed on precision (%CV)

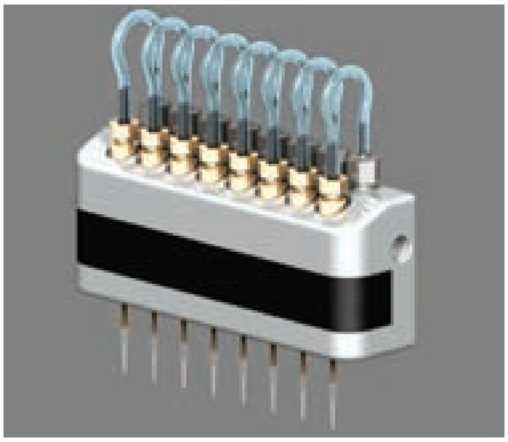


CaliperLS SciClone inL10™ workstation uses a solenoid sensor in each channel

landmark in the development of robust nanolitre dispensing. The first generation instruments (Labcyte's Echo 550 and EDC Biosystems' HTS-01) are focused on supporting compound reformatting applications, but acoustic transducers have the potential to not only add value through eradicating wash steps and eliminating tip utilisation but also by operating the acoustic transducer as a small scale sonar device that is positioned underneath the source plate well. By measuring the time-of-flight of sound energy (the echo return time) to the surface of liquid in the well it is possible to calculate the depth of the well fluid. The amplitude of well fluid bottom reflection, which varies with the fluidic impedance, is then used to determine the fluid composition and the speed of sound. Echoes can then be processed to calculate the DMSO/water ratio and fluid volume. This auditing process can occur even if the wells are sealed or lidded, can be extended to mini and microtubes, and has great potential in quantifying the degree of hydration of compound libraries, originally dissolved on 100% DMSO. **Figure 4** underscores a fundamental advantage of acoustic transfers done with the Echo 550 versus more conventional low volume liquid handling. Acoustics scales drop volumes for transfers by adjusting the wavelength of sound in a liquid and other technologies change something which is tied to the physical dimension associated with the transfer. The lack of sensitivity to dispense volume CVs in Echo 550 transfers results primarily from this decoupling of the transfer process with any solid interaction. The acoustic process scales with wavelength and not with a 'physical dimension' like an orifice diameter or characteristic surface roughness. Sources of variability for 'solid-based' liquid transfer methods arise from geometrical non-uniformity between the dispensing elements (like the orifice size or pin diameter) and surface characteristics of those transfer devices (roughness, coatings, contamination etc). As volumes decrease the variation in these factors are further amplified by the growing impact of surface tension and electrostatic forces. The only 'solid' having a role in acoustic transfers is the well plate bottom, and you can measure its impact on the process by using the transducer as an acoustic microscope. The Echo 550 also takes an active role in reducing electrostatic fields by de-ionising both source and destination microplates. Hence, the remaining source of variability in the process rests in the well fluid samples themselves and that is why the composition and depth detection process is important in reducing the CVs. The viscosity of DMSO increases from ~2 cp to ~3.5 cp as it hydrates from a few per cent to 30% water in a normal laboratory atmosphere. The better the Echo 550 can know how much energy to deliver (from well fluid composition detection) and at what height to deliver it (from the fluid speed of sound and echo traverse time in the fluid), the more accurate the transfer volume will be. Without composition detection, the Echo 550

IMTEK prototype 384 Dispensing Well Plate (DWP™) micro-machined from COC, an integrated dispense and storage device





Aurora Discovery PicoRAPTR piezo dispenser, with close-up of eight individually addressable nano piezo tips (right)

would still retain its ability to dispense the required drop volume, but would see a significant degradation in CVs for all transfer volumes. Further enhancements of the Echo 550 will include the development of more robust methods of temperature compensation as the acoustic impedance of the well fluid and microplate will change with temperature at different rates.

Flow sensors facilitate 'on the fly' dispensing adjustments

The survey also identified a growing interest in solenoid sensor devices, although numerically the numbers of units sold are predicted to be relatively small (possibly up to 50 units by 2005). Potential for growth in sales of solenoid sensor devices like the Sciclone inL10 from CaliperLS, which has an ability to adapt to changing dispensing conditions, can probably be attributed to customer desire to gain further improvements in dispensing quality and reliability and to understand the basis for poor performance (clog detection). The inL10 is the first instrument to offer 100% dispense verification in real time, although the audit trail generated is based on the measurement of flow across a sensor rather than the direct visualisation of deposition in a microplate well. In addition, each channel has a built-in liquid level detection capability as well as a temperature sensor that enables it to automatically compensate for changes in viscosity. Other solenoid syringe dispensing technologies are mainly time-based, so as changes in temperature and viscosity occur, these instruments continue to open their valves and dispense for a fixed period of time. The inL10 does not receive time-based instructions but uses its proprietary MEMS flow sensor technology to open and close the valve in each channel in a dynamic fashion based on closed loop reporting of the actual volume being aspirated or dispensed per channel. In total 109 microprocessors monitor performance parameters across the inL10 96 channel head in real time and facilitate 'on the fly' adjustments to compensate for variations like temperature, viscosity and residual volume that compromise the performance of other approaches, particularly at low volumes.

Integrated dispense and storage devices – hype or reality?

Although HTStec's survey identified considerable interest in integrated dispense and storage devices, like Boston Innovation's Smartplate 384 and IMTEK's Dispensing Well Plate (DWP), neither exists as a commercially available mass produced product and their role in future nanolitre dispensing remains uncertain. Arguably the application both devices initially sought to address, ie combined plate storage and delivery of compounds libraries in DMSO, is one where customers are currently focused on improving quality and are most reluctant to adopt unproven new technologies. For although the highly parallel non-contact nanolitre dispensing potential of full-size 384 prototypes has now been demonstrated the potential of the small orifices and capillaries to clog, the effect of freeze thaw cycles, and the impact compound precipitation on prolonged storage are all significant unknowns. In addition, individually addressable sealed single use mini-tubes stored in 384 plates have emerged over the past few years to compete with plate-based methods of compound storage, offering significant flexibility from a high speed cherry picking perspective. The bulk reagent dispensing potential of IMTEK's DWP either with different reagents to all wells (where each DWP reservoir supplies one nozzle) or the same reagent to all

Reference

1 Comley, JCW (2002). Nanolitre Dispensing – on the point of delivery. Drug Discovery World, 3(3): 33-44.

wells (where all DWP nozzles are supplied by a common reservoir) has yet to be fully explored. The ability of the DWP to repeatedly dispense a fixed nL up to low µL amount to all wells of a 1536 plate in one shot, at a fast (msec) cycle rate, makes it unique among dispensing mechanisms. Also focusing on homogeneous reagent dispensing is consistent with a phased introduction of the DWP. One could imagine a scenario where initially the customer fills the DWP with reagents immediately before use, to make multiple copies, thus gaining technology experience and wider customer acceptance, with gradual progression to reagent storage, with preloaded DWPs offered by a reagent provider as a simple storage/delivery tool and means of minimising the wastage of expensive reagents or those that are more stable in concentrated form. The future of the DWP development now depends on the ability of IMTEK and Enplas (its partner for the production of low cost plastic plates) to find companies interested in the commercialisation opportunities the DWP affords.

Direct dilution curves by volume dispensed

An application that has frequently been discussed in relation to low volume dispensing but never adequately addressed in a commercial instrument is direct dilution, ie making dose-response curves (eg IC50s) on a volume dispensed basis from a single aspirate, rather than by serial dilution within a plate. To undertake such a task requires a low volume dispense capability with a very wide dynamic range, over several logs volume, ideally descending into the picolitre range. The SciClone inL10 supports one-step serial dilution, and although its dynamic range is broad (10nL to 10µL) the lack of a picolitre capability would limit its use in miniaturised assays. Few technologies apart from those based on piezo and acoustic transducer possess this very low end capability. In August 2004 Aurora Discovery will launch its PicoRAPTR with a dispense range from 500pL to 100µL, using piezoelectric dispensing for volumes below 1µL and a positive displacement syringe for volumes above 1µL. The PicoRAPTR features eight individually addressable nano piezo tips. These piezo tips have an injected molded plastic nozzle, a special coating for durability and design modifications to allow for ease of placement/removal from the head. The PicoRAPTR supports dispensing into 96, 384, and 1536 plates and is intended to enable high quality dose-responses curves in high density plates and low volume microplate spotting (down to 500pL). During the conventional serial dilution process compounds frequently come out of solution immediately when diluted with aqueous buffers, with potential impact, usually a reduction, on the true potency of a compound. Direct dilution from stock DMSO into assay buffer should add quality to this process by limiting compound loss during the dilution process. An issue that arises when making direct dose response curves on a volume dispensed basis over a wide concentration range is how to effectively control for the variable volumes of DMSO that will arise in the assay as a result of dispensing variable volumes of drug dispensed from stocks in neat DMSO. The PicoRAPTR's software allows for DMSO correction by calculating total drop count dispensed and then inversely dispenses DMSO up to the maximum volume (highest drug concentration) dispensed in the dose response curve, so all wells receive the same percentage volume of DMSO.

Conclusions

What is evident from HTStec's survey is that users increasingly want robust dispensing solutions, and many users are still reporting that non-contact dispensers cause the formation of bubbles in the wells. Users' expectation regarding the desired performance from a nanolitre dispenser are high and on average want <5% bias dispensing accuracy and <5% CV dispensing precision. Furthermore, users would like to see on board software applications that determine precision and automatically recalibrate a dispenser, together with QC regimes to ensure accurate dispensing performance. In addition, technologies that are seen to add value and enhance application quality are of high interest, but critically it is the price to quality ratio that needs to be right if the technology is to gain the maximum user acceptance.

The author gratefully acknowledges the help of Rich Ellson in putting together **Figure 4**. DDW

***Dr John Comley** is Managing Director of HTStec Limited an independent market research consultancy whose focus is on assisting clients delivering novel enabling platform technologies (liquid handling, laboratory automation, detection instrumentation and assay methodologies) to the HTS environment. Further information on accessing the market report 'Nanolitre Dispensing Trends 2004' can be obtained by visiting www.htstec.com or e-mail surveys@htstec.com to receive a free copy of the Report's Executive Summary and Table of Contents.*