Nanolitre dispensing – a new innovation in robotic liquid handling

With ever increasing pressure on drug discovery groups to provide fast, accurate and concise screening information on potential compounds the emphasis has shifted towards the technology and formats used in screening techniques. Traditionally, the technology has led to the establishment of large, highly automated robotic equipment designed to automate multi-step assays in the 96-well microplate format. The attendant challenges this brings include size, speed of processing and complexity of system.

Recently, several manufacturers of microplates have brought higher density formats to the market – 384-, 864-, 1536-well plates and beyond. The advantages of such formats include a greatly increased number of tests contained discretely within a footprint equivalent to the 96-well format and the provision of many less plates to robotically manipulate to achieve current throughput targets. However, such formats again provide new challenges to the manufacturers of drug screening equipment. Assay volumes have now evolved down to the point where traditional liquid-handling systems cannot accurately dispense the sub-microlitre volumes required. For the development of automated assays on high-density microplate formats, liquid-handling development must keep apace with the requirements of such small volumes. Finally, concomitant to these changes, detection techniques will also need to be enhanced to give greater levels of sensitivity, and systems will be required to parallel process hundreds or even thousands of samples at a time.

As well as high-density microplates, interest is growing in assay technology that can be performed satisfactorily in other formats, most notably capillaries, etched silicon and other microchambers. The ability to pipette small volumes with acceptable precision and accuracy into such receptacles has always been limited by traditional liquid-handling systems. Currently available technologies capable of low-volume dispensing include piezoelectric, thermal transfer and solenoid-valve-actuated methodologies. This article focuses on the PiezoTip, a new piezoelectric automated liquid handling system that has been recently launched by our group at Packard Instruments.

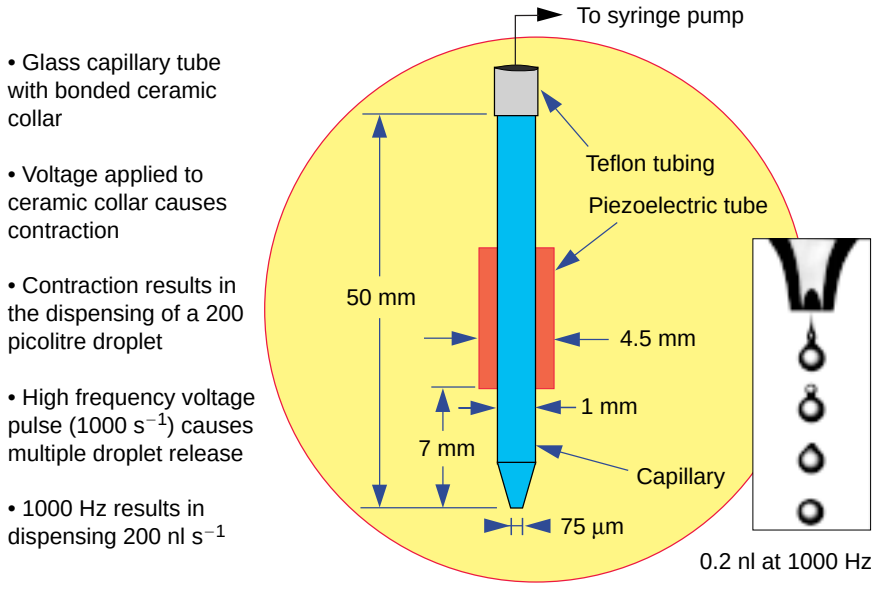

Figure 1. *Principles of piezoelectric dispensing.*

Piezoelectric dispensing

The principles of piezoelectric dispensing are illustrated in Fig. 1. A ceramic collar is bound to a glass capillary, one end of which is attached to a syringe pump through a liquid filled line and the other end is used for dipensing. When a voltage is applied to the ceramic collar, it contracts causing constriction (squeezing) of the capillary and release of a defined volume of liquid. The volume of the 'squeezed' drop is dependent upon several factors, including the diameter of the capillary, the viscosity of the liquid, system pressure, design of the ceramic collar and the intensity and duration of the voltage pulse.

In our system, a single voltage pulse is designed to produce a droplet of ~300 picolitres (pl). With a frequency of 1000 pulses per second, a volume of 0.3 nl s^{-1} will be delivered. The syringe pump attached to the capillary is an integral part of the system. In addition to being used to maintain the pressure of the liquid column during piezo dispensing, the syringe pump can be used for larger volume (µl) dispensing without using the piezo-dispensing mechanism.

By using a proprietary (patent applied for) pressure-sensing technology in conjunction with the syringe pump, the system can verify and control that a droplet has been dispensed; and by monitoring the movement of the syringe the volume of the dispensed sample can be verified. When dealing with droplet sizes that are virtually invisible to the eye, this on-line performance verification is important. To aid this further a videoscope can also be supplied with systems to show, on screen, droplets being dispensed. The use of the videoscope screen has proved to be a valuable calibration aid for the system.

An important aspect of the new piezo-dispensing technology is that it is non-contact. That is, the sample is dispensed without contacting the liquid or surface it is being dispensed into. This prevents cross contamination, increases delivery speed and gives high precision dispensing without touch off.

System availability

The PiezoTip piezoelectric-dispensing technology has been supplied to several

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drug discovery and genomics groups in the USA, UK and Germany.

Currently it is being supplied as an R&D tool for use in assay development and as a validation tool for development groups to gain experience in the aspects of low-volume dispensing protocols. The current system consists of an XYZ robotics stage with four PiezoTip piezoelectric-dispensing probes. The positional capabilities of the system are measured in microns and will allow the probes to service 1536-well formats reproducibly. It is also capable of dispensing onto glass slides, bio-processing microchips and PCR gels. The piezoelectric-dispensing system will be available as an option on Packard Instruments' current range of MultiPROBE liquid-handling systems by the end of 1998. These advances will then be supplemented by further options in system development due to Packard's recent acquisition of the robotic manufacturer Carl Creative Systems.

The combination of piezoelectric-dispensing liquid-handling systems and plate/reaction vessel robotic and storage automation systems will provide powerful tools in the development of ever faster drug discovery automation packages.

Applications in drug discovery

The main application area that is currently being assessed for exploiting the new technology is in the direct dispensing of test compounds into dilution formats.

To overcome the inhibition provided by the dimethylsulphoxide (DMSO) used to solubilize many compounds, stock solutions are often diluted in a matrix by 100-fold or greater. In the very busy compound storage banks in drug discovery environments, much time is spent aliquoting from storage plates to test plates for use in testing. Subsequently these plates are often further diluted at the test bench level to provide the required test concentrations and dose–response curves.

Our new Packard piezoelectric automated liquid-handling system allows the compound bank to provide fully diluted, ready-to-test plates directly to the test area by non-contact dispensing and with no pre-dilution required. This overcomes the traditional problems encountered in such preparations, which are tedious and error prone even when automated including a pre-dilution step. In traditional protocols, compound loss due to serial dilutions, non-specific binding to surfaces and precipitation are often encountered problems. Direct addition of nanolitre volumes from a stock solution to the assay plate eliminates the intermediate dilution steps and the potential problems associated with this process. An example of how this can help practically in the laboratory has been seen at a UK pharmaceutical company. Here the integration of PiezoTip technology to four Packard MultiPROBE robotic liquid-handling systems, used for the production of test plates ready for assay, is calculated to lead to a tenfold increase in tests performed (from 20,000 to 200,000 samples) within the same time period.

Other applications for the system include the dispensing involved in cell-based assays. The performance characteristics for the system can be seen in Fig. 2.

In this example fluorescent dye solution was dispensed into 80 μl of buffer in a 384-well plate and the fluorescence intensity measured. The dispensing was found to be linear from 0.2 nl to >6 nl with good coefficient of variation (CV). CVs of 2% for nanolitre volumes are typical with the new Packard piezoelectric technology.

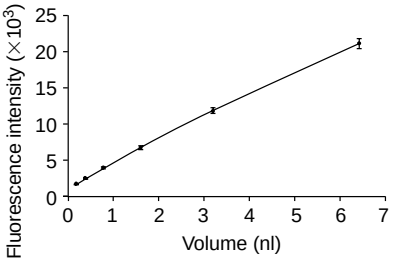

Figure 2. *Linearity of piezoelectric dispensing. Samples of 10 mM fluorescein from 0.2 nl to >6 nl were dispensed into a 96-well microplate containing 80 μl of buffer per well and measured on a microplate fluorometer under standard conditions.*

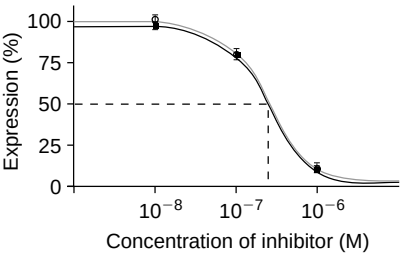

Figure 3. *Comparison of serial dilutions with direct dispensing for transcriptional regulation. An endothelial cell line transfected with the luciferase reporter gene was used to study the inhibition of expression. The inhibitor was added as either a manual serial dilution (circles) or by direct dispensing from 0.2 nl to 25.6 nl using the MultiPROBE (squares).*

A cell-based reporter-gene assay was used to further investigate the benefits of the system in being able to make direct dilutions. The expression of luciferase reporter gene was used to measure growth inhibition in an endothelial cell line.

Figure 3 shows the growth inhibition curves for an inhibitor added either as a 100:1 dilution series or by direct dispensing of the inhibitor from 0.20 nl to 25.6 nl. The inhibition of expression of the luciferase reporter is the same in both methods.

Other applications that have been tested include K562 and MT4 cell-based biological assays that have been dispensed in volumes of 8 nl and above (100–10,000 cells) while retaining their viability. The use of the system in nanolitre dispensing for assays of 1μl or less (total volume) is due for publication soon.

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