Acoustic Droplet Ejection Technology

Acoustic Droplet Ejection (ADE) technology overcomes the analyte transfer problems that have until now blocked advances in the development of low-volume, high-density assays.

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'Miniaturisation' is the mantra repeated endlessly in the drug discovery laboratory. Reducing the volume of assays dramatically cuts costs by saving on expensive and difficult-to-obtain reagents. Typical high-throughput laboratories can save hundreds of thousands to millions of dollars annually by reducing volumes by 90% from 50µL to 5µL. Laboratories also gain from higher sample throughput and reduced space requirements.

BLOCKS TO MINIATURISATION

This being the case, why hasn't everybody switched to high-density, low-volume assays? Many assay reading systems are capable of measuring results in higher density formats; while there may be less total fluorescence, absorbance or luminescence from any single well, this has not been a significant problem. Assay plates themselves are available in formats as dense as 6144-well, and bulk reagents can be added to multi-well plates at the µL level with good precision (<3%).

The remaining problem blocking miniaturisation is transfer of the analyte being tested. When assay volumes are at the 5µL level, the volume of analyte added is usually at the 5-50nL level. Unfortunately, the accuracy and precision of low nanoliter transfers has suffered due to the interaction of the fluid being transferred and the solid interface of the transfer device. Small volumes of fluid cling to irregularities in the surface of the pin tools, pipette tips and nozzles. At microliter levels, these captured volumes have no significant impact on precision or accuracy. As the transfer volume decreases, however, these inadvertent and uncontrollable adsorptions can have a serious impact on precision (as measured by CV) and on accuracy (as measured by deviation from expected). Comley reported that, based upon data supplied by the manufacturers themselves, the CV% for 50nL transferred was typically 4-16%, but rose quickly as the volume dropped further (1). An extrapolation of the data shown in his paper suggests errors of 12-20% or more as volumes decreased to 10nL and lower.

ADE TECHNOLOGY

Acoustic droplet ejection (ADE) eliminates all physical contact between the instrument transferring the fluid and the fluid itself. A pulse of sound is applied to the bottom of a multi-well source plate containing the fluid to be transferred (Figure 1). The acoustic impulse forces a droplet of precise volume to move from the source plate to an assay plate. ADE has been commercialised by Labcyte in the Echo™ 550 and 555 liquid-handlers. These systems transfer individual droplets of 2.5nL, delivering multiple droplets for larger volumes at transfer rates as high as 500 droplets per second. Typical users report CV% of 2-5% at 2.5nL volumes (2, 3).

Nie and Cesarek showed that ADE reduced or eliminated the need for serial dilutions (4). This provides a significant improvement in overall precision for lower concentrations of analytes, as in the case of IC_{50} analyses where researchers may wish to look at the activity of a compound over five or six orders of magnitude of concentration.

DMSO CONCENTRATIONS

As mentioned above, the precision of analyte transfer can be improved with larger transfer. A move towards higher volumes would be a direct retreat from the call to miniaturisation. If the final assay volume is kept low but the volume of analyte transfer is increased, two significant problems can occur. First, when the analyte is dissolved in dimethyl sulfoxide (DMSO), as is usual for high-throughput screening, the percentage of DMSO in the assay itself can become a problem (5). For example, a solution of 0.1% DMSO is 13.8mM; this concentration can be thousands of times greater than the concentration of the analyte being tested. DMSO has been shown to interfere with the binding of analytes to target proteins. A 0.1% solution of DMSO can reduce the amount of ligand-associated protein by 50% and a 1% solution by 90% (6). DMSO can also accelerate enzyme and receptor changes through denaturation and aggregation. In general, therefore, it appears that maintaining a concentration of <1% DMSO is prudent. A 1% DMSO maximum limits the volume of analyte to 50nL in a 5μL assay. To further reduce the concentration to a more biochemically friendly level of 0.1% DMSO or less requires a maximum transfer of 5nL analyte in DMSO for a 5μL assay.

Figure 1: In acoustic droplet ejection, a transducer centred under a well emits a pulse of acoustic energy focused at the meniscus of the fluid in the well

The acoustic pulse precisely and accurately ejects a droplet of fluid from the well. This droplet is captured by an inverted receiver plate positioned above the source well. Because the transducer and the receiver plate can both move independently, the technique provides any-well-to-any-well flexibility. To transfer larger volumes of fluid, the transducer emits multiple pulses. The Echo liquid handlers automatically compensate for variations in the volume of each source well and for variations in the level of hydration of the DMSO solvent.

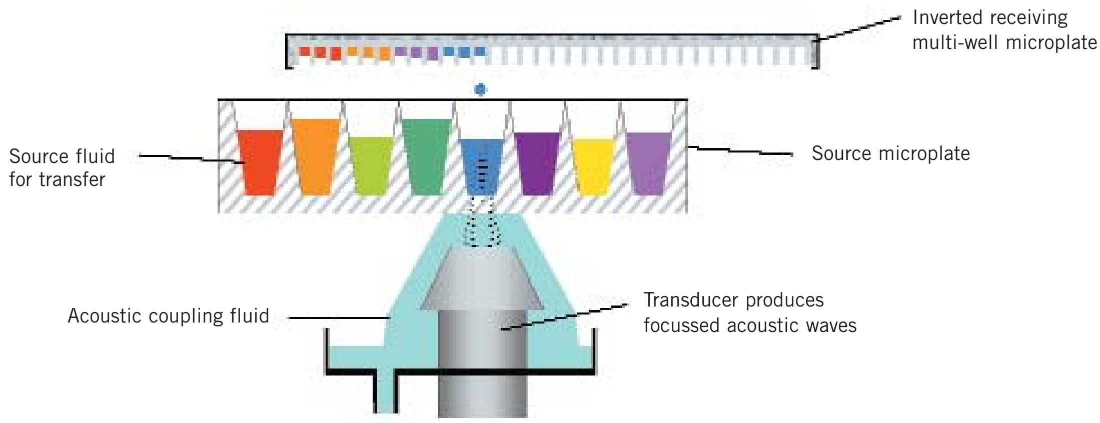
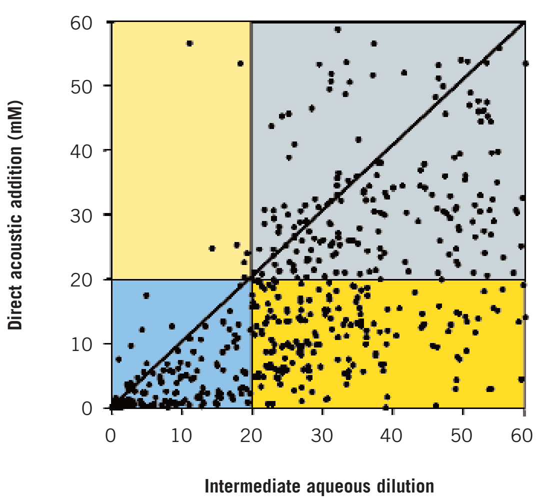


Figure 2: IC_{50} values were measured and plotted for 1,144 compounds using both an aqueous intermediate dilution and with direct ADE-based nanoliter addition

The IC_{50} values obtained from direct ADE addition are plotted along the vertical axis, and the values obtained for each compound via the aqueous dilution are plotted along the horizontal axis. Compounds that had an IC_{50} value of 20µM or less were considered good candidates. The compounds in the blue quadrant were biologically active (that is, IC_{50} value less than 20µM) by both techniques, and the compounds in the grey quadrant were biologically inactive by both techniques. The compounds in the bright yellow quadrant were active when the analysis was performed with direct addition of the analyte via ADE, but appeared inactive when analysed via an aqueous dilution. 110 compounds, approximately 10% of the total, were falsely identified as inactive via the aqueous technique. A number of these compounds falsely identified as non-active had IC_{50} values less than 1µM. The pale yellow quadrant shows six compounds that were identified as active with an aqueous intermediate dilution, but not via direct nanoliter addition. Material adapted from slide 11 of reference 7.



One potential way to reduce the effect of DMSO on the assay would be to dilute the analyte with an aqueous buffer prior to addition to the assay plate. This would allow larger volumes to be transferred with better precision and accuracy. Unfortunately, this dilution step can inadvertently lead to a significant reduction in the amount of analyte actually delivered to the assay. Spicer and co-workers at Bristol-Myers Squibb showed that when an intermediate aqueous dilution was used, numerous compounds (~10% of those tested) registered as false negatives – that is, they were falsely assumed to be inactive (7) (Figure 2). They postulated that the false negatives were due to analytes precipitating out of solution during the aqueous intermediate dilution, thereby reducing the amount of available compound, or due to compounds adsorbing onto the plastic surfaces of the dilution plates or pipette tips.

DMSO can also have deleterious effects upon cells. The negative impact of DMSO upon cells is accentuated by the 'bolus effect' experienced when using traditional pipetting methods or pin tools to add DMSO solutions to cell-based assays (Figure 3). DMSO has a density of over 1.08g/cc whereas the densities of most cell-based assay buffers are closer to 1.00g/cc. When DMSO solutions are dispensed with pipettes or pin tools, the DMSO drops as a bolus, essentially undiluted, to the bottom of the well where the cells are located. The DMSO may solubilise the membranes of cells initiating cell signalling pathways and, possibly, destroying the cells.

When DMSO is added with a pipette or pin tools, the cells are exposed to transient levels of analyte (and DMSO) at far greater concentration than suggested by the concentration of the fully dispersed sample. Visual estimates of dye-loaded DMSO boluses produced by pipettes suggest that the concentration of analyte and DMSO may exceed by fifty-fold the fully dispersed concentration. This situation is different with ADE transfer because the analyte containing DMSO is added to an inverted plate (Figure 1). The denser DMSO spreads along the meniscus of the inverted fluid rather than dropping to the bottom of the well. After loading the analyte, the assay plate is turned right-side up and the DMSO and analyte diffuses into the aqueous solution. When ADE is used, cells can be protected from exposure to high concentrations of analyte and DMSO.

REDUCTION IN WASTE

Finally, as assays are miniaturised and compounds are dispensed by ADE, there will be much less waste generated in the lab. The use of ADE eliminates the need for plastic pipette tips and the DMSO for washing both fixed and disposable tips. Spicer showed that the high-throughput screening lab saved hundreds of thousands of dollars by eliminating pipette tips and DMSO washes by moving to the completely touchless ADE method. The authors estimated savings and reduction in waste generation from switching from conventional processes to acoustic transfer. Data was supplied by customer HTS labs. One sample case study was for a 1.3 million compound library in 384-well plates accessed four times a year to enable eight individual assays each time. This throughput is easily accomplished by a single Echo 550 liquid handler. Disposable pipette tips were re-used 100 times, each with a 0.7mL DMSO/water wash between sample changes, to make intermediate daughter plates and 500 times with 0.9mL DMSO/water wash to make assay plates.

While the savings from elimination of pipette tips, intermediate plates and seals are well recognised, the disposal costs are often not included in an economic analysis. The ADE-based laboratory reduces waste by 208 litres of DMSO solution and 2.8 metric tons of plates and seals. At $25 per kilo for solids and $42 per barrel for liquids, the disposal costs account for 30% of the more than $200,000 per year that can be saved when an HTS line is converted from conventional dispensing to ADE. Even greater savings can be attained by further miniaturisation to the 1536-well format.

CONCLUSION

The promise of low-volume, high-density assays has been delayed by the inability to transfer nanolitre volumes of analytes precisely and accurately. This limitation has been eliminated with the introduction of acoustic droplet ejection, as embodied by the Echo 550 and Echo 555 liquid handlers. Low transfer volumes enabled by the Echo liquid handlers enable reduced assay volumes, while maintaining low DMSO concentrations in the final assay without resorting to aqueous intermediate dilutions. False negatives are reduced and consumable costs drop significantly. Acoustic droplet ejection reinvigorates the mission to realise the potential of HTS assay miniaturisation.

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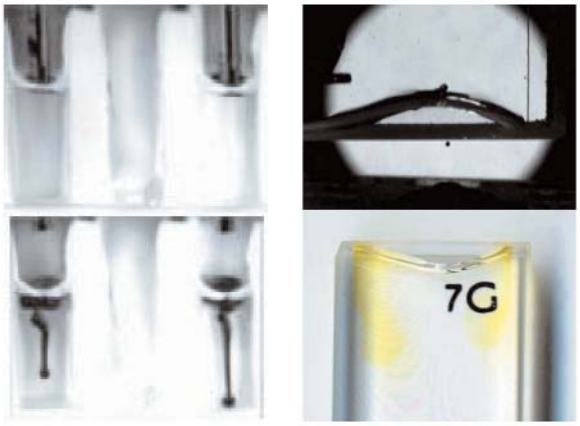
Figure 3

Upper left – DMSO coloured with dye is held above the surface of a liquid in a pin tool. *Lower left* – The pin tool has touched the surface of the liquid in the assay plate and the dense bolus of DMSO drops rapidly to the bottom of the well, exposing any cells at the bottom of the well to high concentrations of both DMSO and any compounds carried by the DMSO. The dye disperses to an equal concentration throughout the well over about 10 minutes. *Upper right* – In an ADE transfer, a small drop is generated and travels upward into an inverted receiver. A collection of drops of the denser DMSO breaks through the aqueous meniscus but then spreads out along the meniscus. This dilutes the DMSO and increases the area in contact with the DMSO. *Lower right* – When the well is inverted, the diluted DMSO disperses into the well along the edges as well as from the entire liquid surface. No plumes of DMSO are seen to reach the bottom of the well. All DMSO appears completely dispersed by the time it comes in contact with the bottom of the well. The pictures of the DMSO bolus from a pin tool are from Reference 8. The movies of the DMSO bolus are courtesy of Dr Joerg Dreessen of Novartis (8).



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