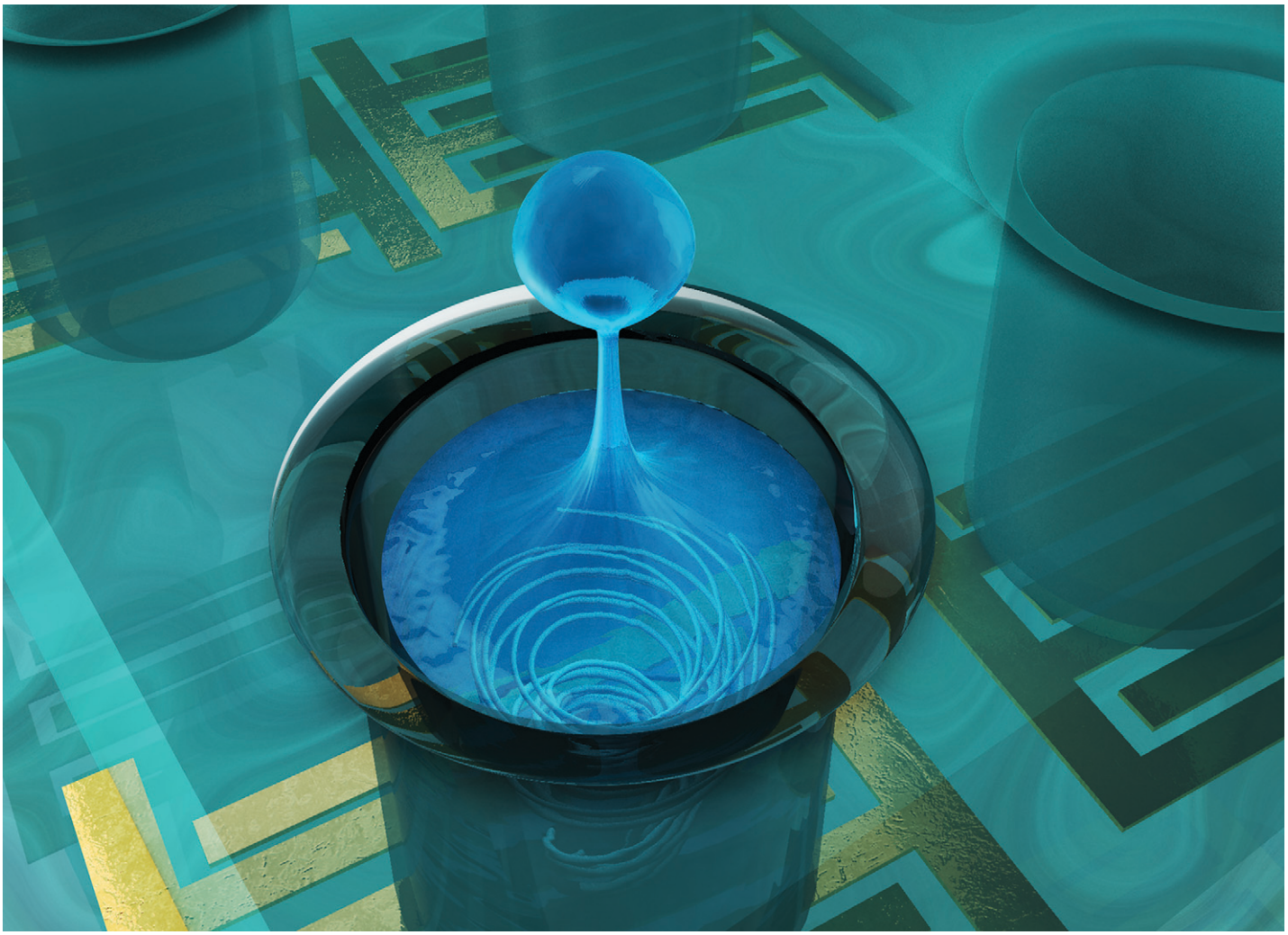
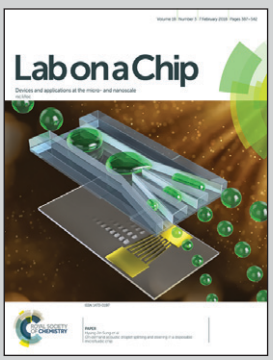


Featuring work from the Micro/Nanophysics Research Laboratory of Professor Leslie Yeo, RMIT University, Australia.

Plug-and-actuate on demand: multimodal individual addressability of microarray plates using modular hybrid acoustic wave technology

Retrofitting microfluidic technology to established laboratory formats and protocols in the drug discovery workflow through a reconfigurable, modular hybrid acoustic wave platform that allows sequential or simultaneous addressability of individual or multiple wells in a microarray titre plate.

As featured in:



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Plug-and-actuate on demand: multimodal individual addressability of microarray plates using modular hybrid acoustic wave technology

Amgad R. Rezk, Shwathy Ramesan and Leslie Y. Yeo *

The microarray titre plate remains a fundamental workhorse in genomic, proteomic and cellomic analyses that underpin the drug discovery process. Nevertheless, liquid handling technologies for sample dispensing, processing and transfer have not progressed significantly beyond conventional robotic micropipetting techniques, which are not only at their fundamental sample size limit, but are also prone to mechanical failure and contamination. This is because alternative technologies to date suffer from a number of constraints, mainly their limitation to carry out only a single liquid operation such as dispensing or mixing at a given time, and their inability to address individual wells, particularly at high throughput. Here, we demonstrate the possibility for true sequential or simultaneous single- and multi-well addressability in a 96-well plate using a reconfigurable modular platform from which MHz-order hybrid surface and bulk acoustic waves can be coupled to drive a variety of microfluidic modes including mixing, sample preconcentration and droplet jetting/ejection in individual or multiple wells on demand, thus constituting a highly versatile yet simple setup capable of improving the functionality of existing laboratory protocols and processes.

Gene, protein and cell analysis workflows for target identification in drug discovery and development[1,2] often consist of an arduous series of complex parallel liquid handling protocols, including a combination of sample dispensing, dilution, mixing and/or pre-concentration steps within the wells of a microarray titre plate, and potentially, the subsequent transfer of the sample out of the wells for further separation and analysis. Conventional liquid handling technologies primarily employ robotically-actuated micropipetting, although the use of pipettes not only poses contamination risks and are limited by the submicrolitre volumes they can handle, but are also prone to error and 'silent' mechanical failure,[3] which can too often be challenging to detect in a timely manner.[4]

Micro/Nanophysics Research Laboratory, School of Engineering, RMIT University, Melbourne, VIC 3000, Australia. E-mail: leslie.yeo@rmit.edu.au

Non-invasive or pipette-free technologies such as microfluidics have thus long been regarded as attractive alternatives to the microarray format.[5] Nevertheless, despite considerable advances in microfluidic platforms for genomic[6,7] and proteomic[8,9] screening, cell analysis,[10,11] and combinatorial drug testing[12,13] in the past decade, the ubiquitous microarray titre plate remains a stalwart in high throughput drug screening and biochemical analysis. This could partly be due to the aversion of laboratory practitioners to new technology or protocols, which are often perceived as unnecessarily complex, even if they are more efficient or cost effective. Alternatively, this could simply be due to the compatibility of existing equipment and methods with the array of ancillary technology such as microplate readers and microscopes that are already available in the laboratory, so as to avoid the need to invest in the infrastructure costs and training resources associated with the procurement of new equipment to accommodate new formats and protocols.[14,15]

As such, there have been parallel efforts to interface non-invasive liquid manipulation methods with microarray technology beyond the range of conventional orbital shaking, magnetic stirring and ultrasonication microplate mixing technologies available, which typically vibrate the entire plate and therefore do not allow individual well addressability. An example is the bulk ultrasonic transducers used for the transfer of nanolitre sample liquid volumes *via* acoustic jetting to, from or between the wells.[17] To individually address a well on the microarray titre plate, the transducer has to be positioned under it using a robotic slider. Such a setup, however, mechanically limits the operation to sequential steps where each well is addressed one at a time.[18] Unless multiple positioners and robotic sliders are employed, which significantly drives up equipment cost, size and complexity, the benefits of parallel handling exemplified by robotic micropipetting cannot be replicated, thereby considerably hampering sample processing times and hence overall throughput. In addition, these acoustic liquid handling systems (Echo®, LabCyte Inc., San Jose, CA, USA) have been limited to liquid

dispensing to date—other sample manipulation modes such as mixing and/or pre-concentration have yet to be demonstrated with this technology, let alone the possibility of actuating a combination of these modes with the same platform.

More recently, surface acoustic wave (SAW) microfluidic technology,[19–23] which has emerged as an efficient means for driving chipscale particle[24–30] and droplet[31–33] manipulation, microchannel actuation,[34–38] mixing, and particle concentration,[39–41] have been proposed as a means for directly interfacing the microarray titre plate.[16] Nevertheless, individual well or simultaneous multiwell addressability on demand is still not possible using this technique, which is employed by the PlateBooster™ system (Advalytix AG, Munich, Germany), since all of the wells directly in the path of the SAW transmission are excited by the acoustic wave, as illustrated by the example in Fig. 1(a). For this reason, it was proposed that individual SAW chips (single crystal 128° *Y–X* lithium niobate) be interfaced beneath each well,[15] although this in practice is constrained by the necessity for interdigital transducer (IDT) electrodes on the top face of the chip to generate the SAW. Given that the IDTs occupy considerable space on the chip to the extent that they interfere with neighbouring wells, even for the larger 24-well plate format (Fig. 2(a)), this physical constraint jeopardises the addressability of all individual wells in the entire microarray plate.

Eliminating the use of IDTs by employing a plate electrode to drive bulk acoustic waves on individual piezoelectric discs directly beneath each well[42–44] has also been proposed,[45] although the need for electrical connections on the top and bottom faces of the discs under each well imposes considerable difficulties in wiring each individual disc; in fact, a single wire connecting multiple discs was instead depicted in that work (Fig. 1 in ref. 45), which would have led to activation of the entire row of wells above the discs connected by the same lead, resulting in the same limitation as the case illustrated in Fig. 1(a). As such, while proof-of-concept of actuating the liquid in a single well was shown, the possibility of individually addressing all of the wells on the microarray plate was never demonstrated, let alone on industry-standard 96- and 384-well plate formats. Additionally, it is necessary for the piezoelectric transducer in ref. 45 to be mounted at an inclination angle with respect to the well plate, which not only complicates the setup, manufacture and assembly of the platform but also limits accessibility to the rest of the wells for individual addressability.

To circumvent these fundamental limitations, we have developed a modular and reconfigurable platform that utilises individual chips whose dimensions completely match the well dimensions, as illustrated by the schematic in Fig. 1(b), so that each well can be directly and individually, or even simultaneously, addressed on demand without incurring crosstalk of the signal with neighbouring wells exemplified in Fig. 1(a). The miniaturisation of the chip dimensions without loss in efficiency is made possible by patterning the IDTs on the *underside* of the chip and employing a recently discovered class of sound waves known as *surface reflected bulk waves* (SRBWs) generated on a HYbrid Resonant Acoustic (HYDRA) platform[47] in which the chip thickness ($h \approx 500$ μm) is matched to the wavelength, set by the width and gap of the IDT patterns, which, in turn, specifies the resonant frequency—here, 10 MHz—at which the IDT is excited. Unlike SAWs, which are only generated and propagate on the face of the device on which the IDTs are patterned (in this case, the underside), these hybrid surface and bulk waves are generated on the IDTs but propagate through the thickness of the chip to the top surface,[47] where they interface with and are transmitted into each well (Fig. 1(b)). In this way, it is possible to reduce the chip dimensions to that comparable

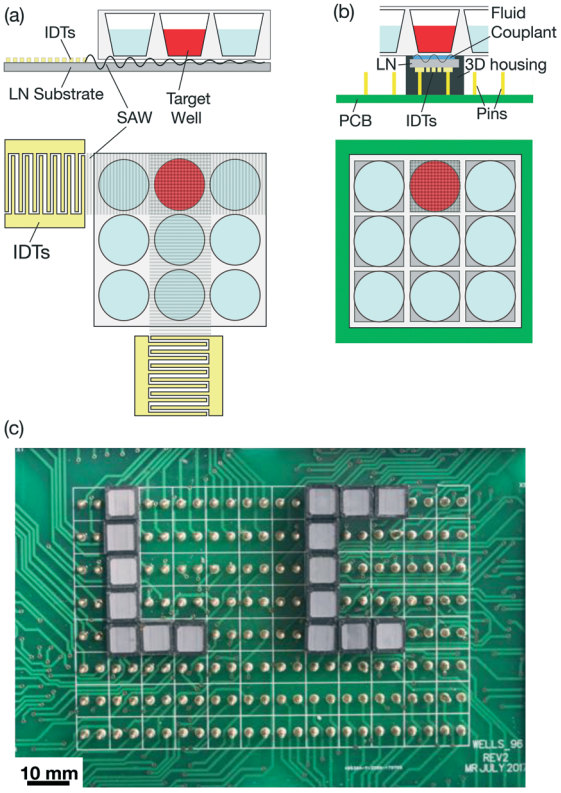

Fig. 1 Schematic illustrations depicting two different principles by which (a) SAWs, and, (b) SRBWs in the present HYDRA device, can be coupled from a piezoelectric lithium niobate (LN) substrate to individually address a target well (shown in red) in a microarray plate. (a) In a system not dissimilar to that employed by the Advalytix PlateBooster™,[16] liquid manipulation in the target well can be driven by exciting two orthogonal SAWs with the aid of a pair of IDTs whose transmission paths intersect beneath the well. It can however be clearly seen that addressability of a single well is not possible since entire rows of wells in the transmission pathway of the SAWs (shaded) are also concurrently excited. (b) In the present system, addressability of a single target well is achieved by mounting standalone LN chips beneath each well that possess IDTs on their underside which are electrically connected by plugging (c) the chip modules onto a PCB *via* soldered pins. The SRBW that is generated on the underside of the chip where the IDTs are patterned propagate through the thickness of the chip to the top face where they are transmitted into the wells. Not only can each well or multiple wells be addressed in this manner sequentially or simultaneously, the modules can also be arbitrarily arranged to flexibly support any desired well or microarray plate configuration, as shown in (c).

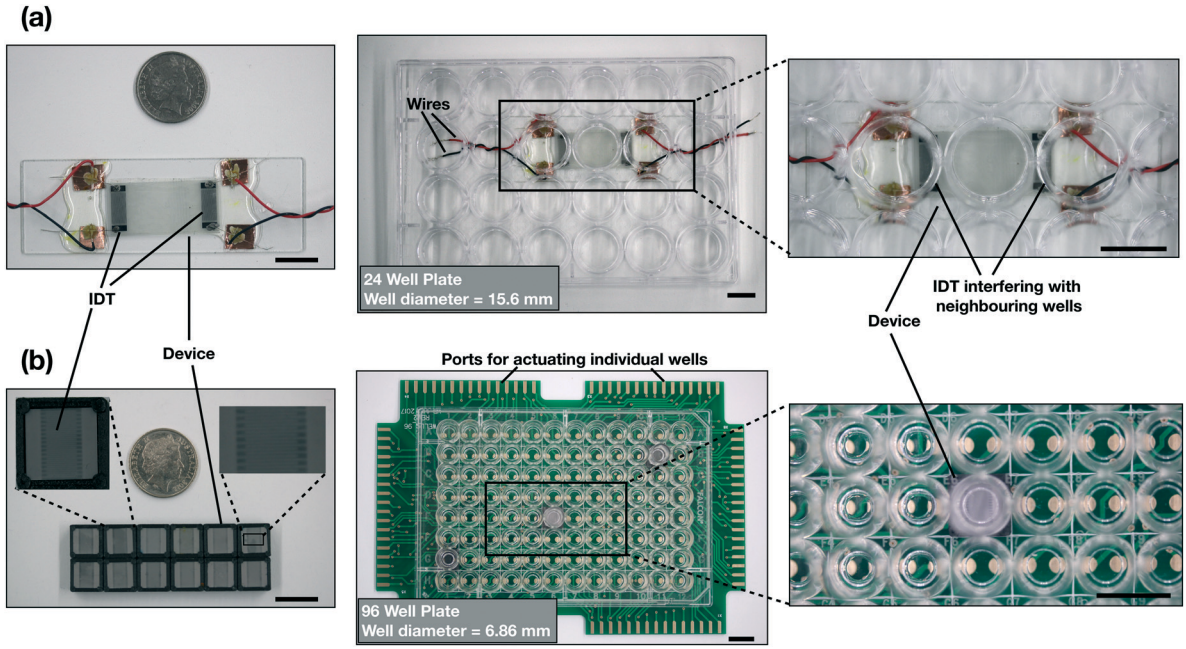

Fig. 2 (a) Top view images of a SAW device (left) interfacing with a well in a 24-well microarray plate (centre). The magnified view on the right clearly shows the interference of the acoustic wave generated by the IDTs with neighbouring wells, thus highlighting the inability of the device to facilitate individual addressability of all the wells on the plate, and the limitation encountered when attempting further size reduction beyond the 24-well plate format. (b) Top view image of the much smaller modular HYDRA devices (left; the image of the devices are flipped to show the IDTs on the underside), each of which can be mounted beneath every single well on a 96-well plate (centre) and electrically connected to a PCB from beneath; for clarity, only one module has been plugged into the PCB. The magnified view on the right shows the possibility for individual addressability of each well or even simultaneous addressability of multiple wells on demand since the devices are not only matched in dimension so that they only transmit acoustic energy into the well that is directly above them, but are also isolated from neighbouring devices by the 3D printed housing that encases them. The scale bars denote a length of 10 mm.

with the IDT dimensions since no additional surface area for the transmission of the acoustic wave is required on the chipface. This then allows us to exactly match the dimensions for a 96-well plate as shown in Fig. 2(b), although we envisage the possibility for further scaling down the chips to accommodate the additional wells in a 384-well plate given that the IDT dimensions are fundamentally limited only by the acoustic wavelength.

Moreover, the placement of the IDTs on the underside allows us to circumvent the limited space available for electrical connections that have plagued preceding technologies (see, for example, Fig. 1(a) and ref. 45). This is because we are able to directly access the IDTs from below by snap fitting each chip, mounted in a 3D printed housing, onto each of the 96 protruding connection pin pairs soldered on the custom-designed printed circuit board (PCB) platform shown in Fig. 1(c), on which traces for the electrical excitation of each individual well are linked to edge connectors at the periphery of the PCB (Fig. 2(b)). These can then be manually or digitally triggered by switches controlled by an Arduino board. Further, the modular nature of the devices allows flexible reconfiguration of the platform to accommodate widely different formats beyond the standard microarray plate, as exemplified in Fig. 1(c).

We now turn to demonstrate the capability of the modular HYDRA platform for on-demand addressability of individual wells to carry out a number of typical liquid handling processes required in the microarray workflow, such as sequential mixing, particle/cell concentration, and single droplet ejection from single or multiple wells *via* liquid jetting—such a capacity to carry out a combination of these modes on the same platform is an advance over many current technologies, which are limited to carrying out only a single operation. Fig. 3, for example, shows the possibility of driving on-demand mixing of a small quantity of blue dye which was deposited with the same quantity into each of the 96 wells on the microarray plate that initially contained a pink-dyed solution. The ability for individual addressability can both be seen in Fig. 3(a), which shows the mixing to be arbitrarily actuated only in wells that were excited with the SRBW, whereas Fig. 3(b) and (c) show the possibility of sequentially addressing these individual wells. That negligible mixing is apparent in unexcited wells adjacent to those that were excited suggests minimal crosstalk of the acoustic wave between neighbouring devices as well as crosstalk of the vibrational signal between neighbouring wells—a problem which besets the setup shown in Fig. 1(a) and 2(a) where the piezoelectric substrate spans neighbouring wells or even many wells. In the mixing operation, we estimate the power loss due to impedance mismatch during transmission of the acoustic energy from the device through the fluid couplant into the well to be up to approximately 50%.

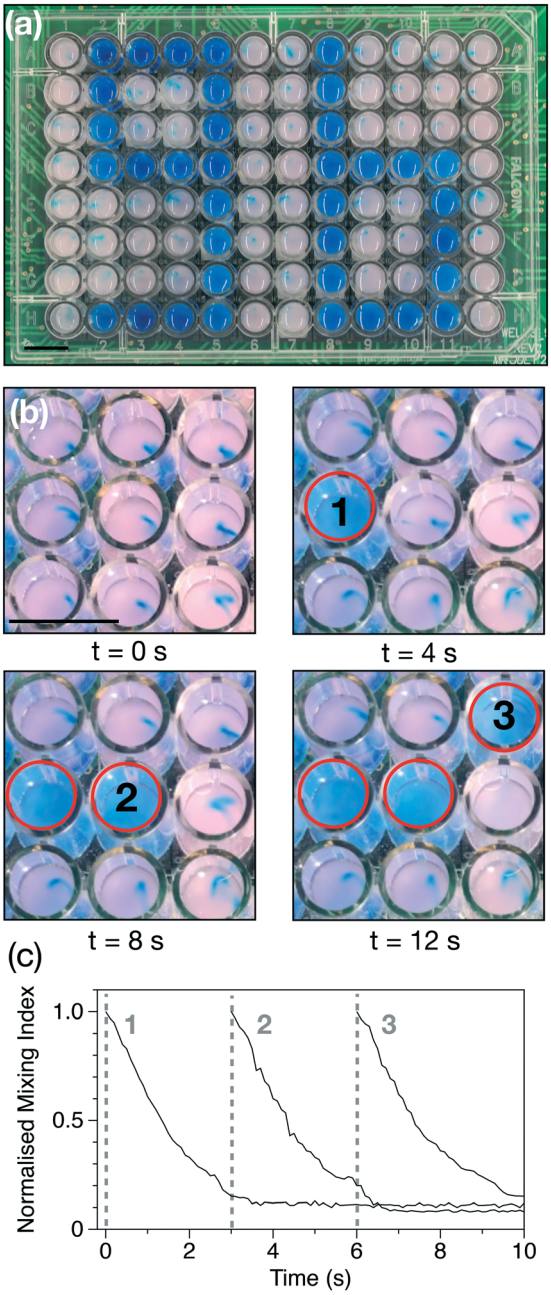

Fig. 3 (a) Simultaneous, and, (b and c) sequential on-demand mixing in individual wells of a 96-well plate driven using the modular HYDRA platform. Prior to the excitation of the SRBW under select wells, each well contained the same amount of pink-dyed solution (100 μl) into which an equal amount of blue dye (1 μl) was placed. The acoustic excitation beneath wells 1, 2 and 3 were triggered at 0, 3 and 6 s, respectively, as shown by the vertical dashed lines. The normalised mixing index in (c) was calculated in the same manner as that described in ref. 40; a value of 1 denotes the completely unmixed state and a value of 0 denotes a completely mixed state. The scale bars represent a length of 10 mm.

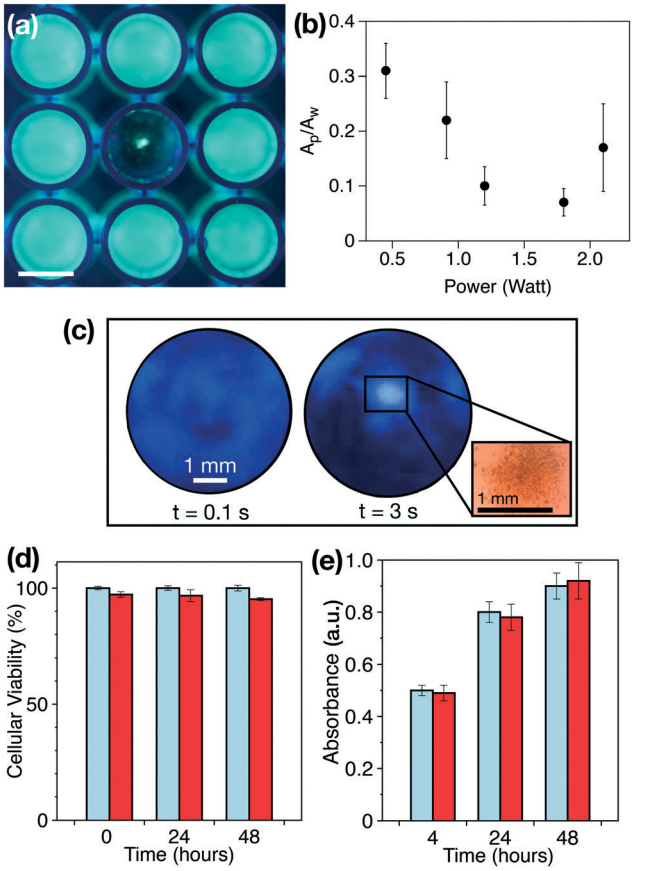

Fig. 4 (a) Top view image showing the rapid concentration of a suspension of 11 μm fluorescently-labelled particles in a select well on a 96-well plate with the modular HYDRA platform within 4 s. Only the central well is excited from beneath with the SRBW in order to induce a microcentrifugation flow within it that drives the particle concentration process. (b) Particle concentration efficiency, defined as the ratio of the area occupied by the particles A_p to the area of the well A_w in the image, as a function of the applied power to the device. (c) Top view image showing the rapid concentration of HeLa cells in the same select well on a 96-well plate using the same platform within 3 s. (d) Viability, as measured using a trypan blue assay, and, (e) proliferation, as measured using a MTT assay, of the HeLa cells, immediately and after 24 and 48 hours following their concentration under the acoustic excitation in the central well compared to cells in the neighbouring well which were not acoustically excited. In (e), the proliferation of the cells are quantified by the absorbance at 540 nm of dissolved formazan crystals converted from the MTT reagent by actively proliferating cells. The protocols for the trypan blue and MTT assays are described in ref. 46.

Fig. 4, on the other hand, shows the possibility for inducing microcentrifugation and hence particle/cell concentration in individual wells on demand. It can be seen from Fig. 4(a) and (b) that the suspension of polystyrene particles housed in the central well is rapidly aggregated into a tight cluster within 5 s upon excitation of the SRBW beneath that well; the mechanism by which the azimuthal microcentrifugation flow arises, which, in turn, drives the particles to concentrate, has been discussed previously elsewhere.[48] As previously observed, an optimum power exists for maximum concentration efficiency; above this value, the increase in the acoustic streaming intensity within the well begins to disperse the particles, thus destabilising the particle clusters and therefore leading to more inefficient concentration.

That the liquid in all of the neighbouring wells, which contained the exact same suspension and which were not

excited, remained quiescent and that the particles remained dispersed, again testifies to the ability for on-demand single-well addressability without crosstalk with neighbouring wells. The ability of the platform to rapidly drive the clustering of cells, which has been shown to be a precursor step for spheroid formation,[15] within a specified well is shown in Fig. 4(c). We note that despite the temperature rise of the transducer of up to 20 °C, the short exposure duration (seconds) of the wells to the acoustic excitation means that the temperature rise of the liquid inside the wells does not exceed 4 °C. We verified that the cells remained unaffected by the exposure to the acoustic excitation, as can be seen from the results of the short and long term cell viability and proliferation tests in Fig. 4(d) and (e), respectively.

Finally, we demonstrate the possibility of extracting a small volume of liquid from individual wells at will—such an ability for external sample transfer being useful for sampling individual wells for further separation or analysis. Fig. 5(a) shows the formation, elongation and subsequent pinch-off of a liquid jet[49] within a well when subjected to an acoustic wave pulse from beneath to form a single droplet, which, in turn, is ejected from the well; the jetted droplet size can be tuned by varying the power applied to the device (Fig. 5(b)). In addition, successive droplet ejection from different select wells can also be effected by sequentially triggering SRBW pulses under each well, as shown in Fig. 5(c). Given the modular setup, the flexibility to simultaneously drive these events from multiple wells or sequentially from the same well can also be easily envisaged, which constitutes a far more attractive alternative compared to the slow and complex process of mechanically moving a single ultrasonic transducer to actuate different wells in the LabCyte Echo® system.

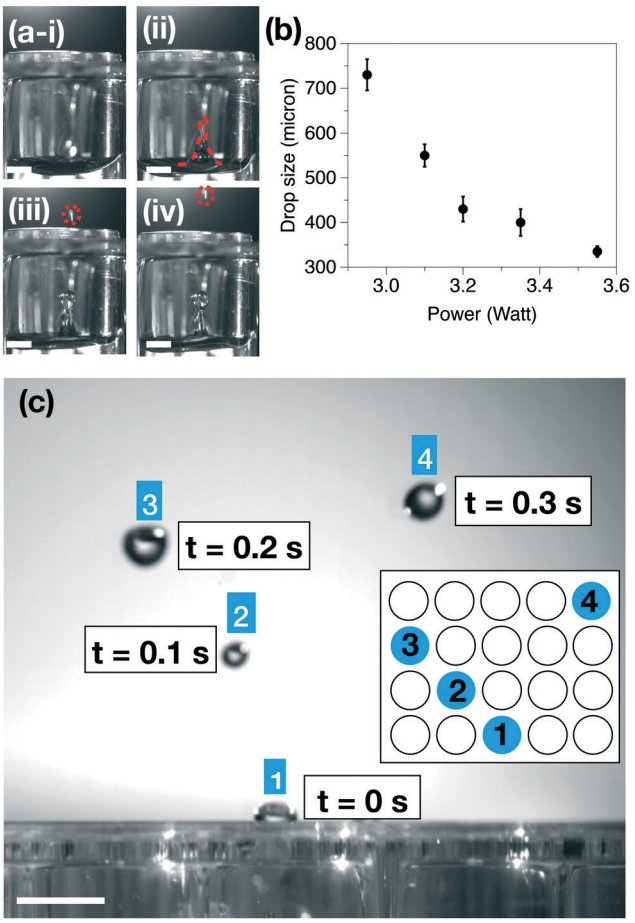

Fig. 5 (a) Time lapse images showing the formation of a liquid jet in a well excited from beneath by the SRBW, and its subsequent pinching to form a single droplet that is then ejected from the well. The dashed lines were added along the liquid interface to guide visualisation. (b) Ejected droplet size as a function of the applied power; the initial liquid volume in the well was 200 μl and a pulse duration of 7 ms was maintained across all experiments. (c) Sequential single droplet ejection from the different select wells shown in the inset by successively triggering a SRBW pulse under each well at 0.1 s intervals. Each ejected droplet consisted of approximately the same volume (700 ± 50 nl). The scale bars denote lengths of approximately 2 mm.

In summary, we have demonstrated a versatile modular plug-and-actuate concept that is truly compatible with the ubiquitous microarray titre plate. This novel platform is capable of efficiently driving a range of microfluidic actuation processes from mixing, sample preconcentration and external liquid transfer—all of which comprise critical steps in the drug discovery workflow—on demand, with the possibility of addressing individual, multiple or all wells on the plate sequentially or simultaneously, thus constituting a significant step towards improving the functionality associated with existing laboratory protocols and processes.

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Conflicts of interest

There are no conflicts to declare.

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