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Picoliter: enabling precise transfer of nanoliter and picoliter volumes

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Picoliter has a technology for the precise transfer of picoliter and nanoliter volumes of liquid without contact between the device and the fluids being transferred. This is accomplished using focussed acoustic energy that causes droplets to be ejected from a liquid surface. Applications include liquid transfer devices, particle manufacturing, and the production of arrays with probes of any length.

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▼ Picoliter has developed a novel microfluidics technology to enable precise movement of small liquid quantities. The total cost of purchasing or producing reagents in the pharma industry is rapidly increasing because more tests can now be performed using highly automated equipment. For many laboratories, reagents have become the largest single expense now that automation has reduced workforce expenses and continuous operation cuts equipment depreciation on a per-test basis. Efficient use of reagents and test samples therefore offers one of the best potentials for cost savings in these laboratories.

Technology

Picoliter™ liquid handling technologies use acoustic energy to move small amounts of liquid precisely. A transducer converts radio frequency (RF) electromagnetic energy into physical vibrations to supply the acoustic energy for this transfer (Fig. 1). This transducer does not contact the source fluid, the droplets being transferred, or the receiving surface. This is a unique feature of the Picoliter technology and is in contrast to other approaches, such as those using piezoelectric (inkjet) devices, which are commonly referred to as 'non-contact' dispensing. Although these 'non-contact' devices do not contact the receiving surface, they do require contact with the pool of liquid from which the sample is withdrawn and therefore require either washing steps or disposable pipette tips when used with multiple fluids.

Picoliter technology has broad applicability in terms of the size of the volumes of fluid transferred as well as the types of solutions handled. Volumes between 0.1 pl and 1 µl have been transferred as single droplets, and these droplets can be ejected from small initial volumes. For example, Fig. 2 shows a 20 pl droplet being ejected from a 20 nl initial volume. Transfer of volumes in excess of one microliter can be accomplished with Picoliter technology using multiple drops, or can be easily achieved with traditional technologies. We have measured the volume of these drops by transferring thousands of drops and measuring the total ejected volume, as well as by calculations based on drop diameter from digital stroboscopic images calibrated against precision micrometer stages. Drop volume consistency was determined indirectly by the size of spots formed on a substrate, which is then scanned at high resolution. Spot size variability is under 2% for 100 µm diameter spots scanned at 5 µm resolution.

We have shown that a wide variety of different materials of biological interest, including water, acetonitrile, dimethyl sulfoxide (DMSO), glycerol–water mixtures, live cells, DNA and proteins can be transferred with acoustic energy. DMSO is especially important because it is used for the libraries of potential drug candidates in pharmaceutical companies. Acetonitrile is a suitable solvent for spotting synthetic DNA onto arrays. We have transferred cells (for example,

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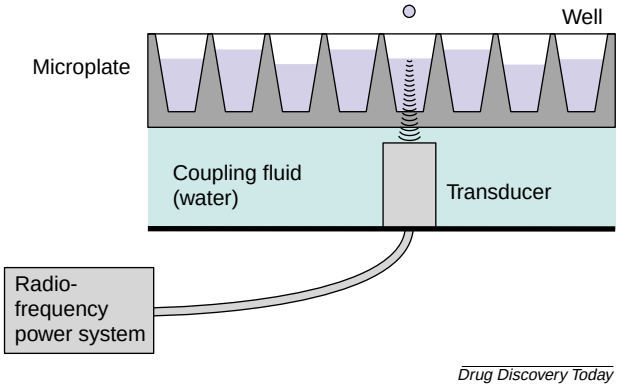

Figure 1. Focussed acoustic energy is generated by a transducer located outside of a conventional well plate. When the energy traverses the bottom of the plate and comes to a focus near the surface of the fluid in the well with sufficient strength, the surface is disrupted and emits a droplet. Droplets are directed towards a porous surface to produce arrays. For liquid transfer applications, the droplets are transferred into an inverted microplate. In both cases, the droplets are easily retained by surface tension.

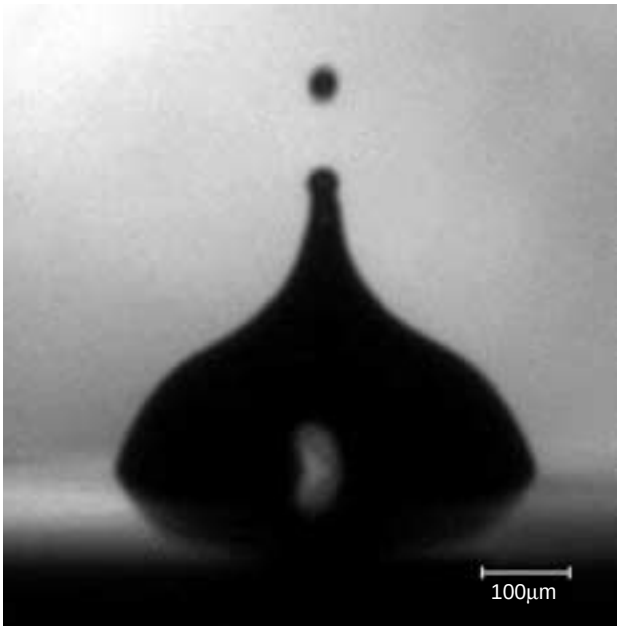

Figure 2. Picoliter technology enables ejection of small droplets from correspondingly small initial volumes and without any solid contact between the transfer liquid and the transfer mechanism. Here, a 20 pl droplet is shown emerging from a 20 nl volume on a flat surface.

human fibroblasts, human kidney epithelial and HeLa) without detectable loss of viability, and enzymes [for example, Taq polymerase, green fluorescent protein (GFP) and lysozyme] without loss of activity. We have not observed DNA degradation caused by the process and have transferred DNA as large as 48 Kb DNA repeatedly.

Products

Picoliter technology enables the development of a wide range of products, including production of medium and high-density microarrays, as well as instruments for precision liquid transfer in HTS and other high-throughput biology applications. The company's first products will be high-density DNA microarrays that satisfy the currently unmet need for a high level of batch-to-batch consistency, and the ability to use probes of any length. Picoliter technology can also be used to create arrays of proteins, protein-capture reagents and small molecules. As these materials become available, the array product line will expand beyond DNA arrays. Picoliter expects to produce arrays in 2002.

The company's next products will be instrument systems that enable the routine use of 384-, 1536- and 3456-well microplates that will decrease the amount of reagent required for each test by a factor of 30 to 400. Although most assays have sufficient sensitivity to be used in these high-density formats, those with more than 384 wells are uncommon because of the difficulties in transferring reagents into these formats. The use of these higher-density plates in HTS applications will dramatically increase the number of assays that can be performed on the limited amount of each test compound and thereby enable screening against the 2000–4000 new targets being discovered through genomics.

All existing liquid handling systems, such as pin spotters, plastic pipetters and piezoelectric devices, require contact between the solid surface of the device and the liquid being transferred. This requires either costly disposable pipette tips or time-consuming washing steps. There is always the concern that washing is not completely effective and that subsequent assays are contaminated by carry-over. The Picoliter device has no contact with the solution, and so disposables or washing are never required, ensuring there is no chance of contamination.

Additional products that will be developed include systems for dispensing cells into cell-based assays, which are now preferred because of their superior biological relevance. The gentleness of the acoustic transfer process makes it especially appropriate for this application. The company expects to introduce fluid transfer and cell dispensing instruments in 2003.

The Picoliter technology also has applications in specialized particle manufacturing for production of pharmaceuticals and cosmetics as well as for life science reagents. The company is

looking for licensing and collaboration opportunities that will enable it to gain value from applications outside of the life science tools area.

Company background

The company is managed by experienced professionals with backgrounds in the computer, high technology equipment and biotechnology businesses. The Chief Executive Officer and Chairman, Elaine J. Heron, has 18 years' experience in the life-science tools industry and was most recently the General Manager of the Molecular Biology Division of Applied Biosystems. The President and Founder, Nannette Simpson, has 18 years' experience in technology development and management and was with Sun Microsystems for ten years. The Chief Technology Officer and founder, Richard Ellson, is a fluidics expert with 20 issued patents, and experience gained at the Eastman Kodak Company and Xerox PARC. Employees of Picoliter have a total of 63 years' experience in the acoustics field and have a total of 34 issued US patents in the area. We currently have 20 employees and a 10,000 ft^2 facility in Mountain View, CA, USA.

Initial funding of US$5.3 million was provided by the founders, Delphi Ventures and Alloy Ventures. Delphi is represented on the Board of Directors by Paul Auerbach and Douglas Roeder. Alloy is represented by Craig Taylor. Management is represented on the board by Elaine Heron, Nannette Simpson and Richard Ellson.

Conclusions

Technology advances are required for continued advancements in life-science research and drug discovery. In order to meet the demands for improved human health, scientists need: (1) higher quality and more reliable experimental data; (2) increased efficiency and throughput of the entire R&D process; and (3) direct cost reductions. Picoliter will directly address the key cost reduction and quality improvement requirements by providing DNA arrays with unmatched product consistency and quality, and by enabling precise nanoliter and picoliter liquid transfer that will produce significant reductions in the amount of reagent used per data point. Picoliter is uniquely positioned to provide products that are in perfect alignment with the growth drivers in life-science research and development.