Novel Acoustic-Wave Micromixer device for mixing, solubilization, and isothermal thawing of microplates

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MICROSONIC SYSTEMS™

Overview

There is an unmet need in the Laboratory Automation industry for effective mixing, solubilization, isothermal thawing and bead and particle resuspension in a microplate format.

Novel Fresnel Annular Sector Actuator-based (FASA) micromixers are designed using Micro Electro Mechanical Systems (MEMS) technology to generate strong Lateral Ultrasonic Thrust ™ (LUT) waves in liquid, creating very effective mixing.

These micromixers have been built into an array of 384 elements to effectively introduce Lateral Ultrasonic Thrust into all wells of microplates simultaneously.

This array of micromixers has been productized into the HENDRIX SM100 to meet the laboratory automation industry's need for effective fluid processing.

Introduction

Mixing has proven to be a limiting factor in the laboratory automation industry's migration to smaller well volumes and higher density plate formats for compound and assay microplates. As the plates move to higher densities, the aspect ratio of the wells, the surface tension of the fluid, and capilary forces all make conventional mixing a more difficult task.

While methods exist for compound solubilization in glass vials, effective solubilization after transfer into microplates has remained elusive. Compound precipitation in these microplates after freeze thaw cycles can cause the effective concentration of the compound in solution to decrease, potentially causing missed active compounds during testing.

Isothermal thawing of liquids changes the state of the liquid without heating. Heating the plates to thaw the liquids inside them can break down the compounds in the wells.

The ability to keep bead, cells, and other particles in suspension in a solution allows for accurate dosing during transfers. The ability to keep cells in suspension during transfer for accurate dosing without affecting their viability is crucial to many cell-based assays.

Technology

The Micromixer Cell

Figure 1 shows the MEMS-based micromixer cell, which is the fundamental element of the LUT transducer.

These elements are made from a piezoelectric material sandwiched between two electrodes. When RF power is applied between the electrodes, the piezoelectric material resonates, generating ultrasonic waves.

These waves propogate vertically in the fluid and impart lateral motion into the fluid.

Fig. 1 MEMS-based micromixer cell

Lateral fluid motion computer simulation models

Computer simulations were performed to model the lateral fluid motion generated inside the liquid using the micromixer cell. These simulations demonstrate how the novel effect of LUT is generated.

Figure 2 shows the simulation results of particle displacements (in r and phi directions in the cylindrical coordinate system) at the designed plane from the micromixer cell which covers a quadrant of a 5 mm radius circle.

These simulations help us to create a vector-based simulation model of the particle displacement.

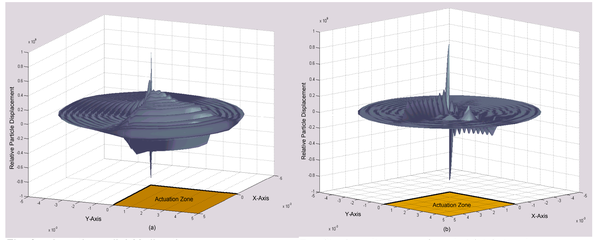


Fig. 2a along the radial (r) direction

Fig. 2b along the circumferential (phi) direction

Simulated Vector Particle Displacement

Fig. 3 shows a simulated vector flow of particle displacement on a plane above the micromixer cell. From this simulation, we observe two loops and the directional shoot-out of the fluid at the center of the figure (i.e., the corner of the micromixer cell).

The flow pattern near the center is very strong, and dominates the fluid flow in the area.

This simulation shows that the main flow is separated into two flows with opposite directions. These flows create the pattern for efficient micromixing.

This vector flow of particles is the LUT Technology that enables efficient mixing in microplates.

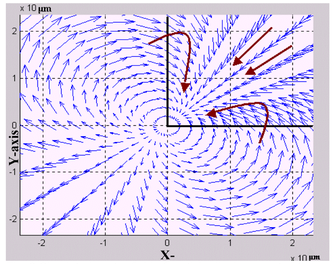


Fig. 3 Simulated vector field of particle displacements at a plane above the micromixer cell.

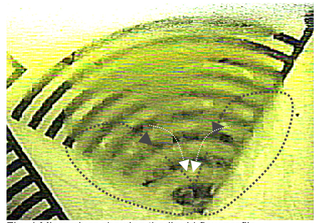
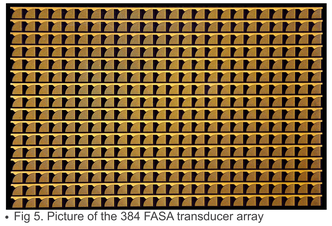


Fig. 4 Micromixer showing the liquid flow profile

Experimental Particle Displacement

Figure 4 shows a snapshot over a working micromixer element, demonstrating the liquid flow profile in accordance with the vector field shown in Fig. 3.

The beads mixed here are similar in size and density to many beads used in the laboratory.



- Fig 5. Picture of the 384 FASA transducer array

384 Micromixer Array

The micromixers were built into an array of 384 discrete elements.

The acoustic-wave energy from these elements penetrates the bottom of the microplates and reflects off the surface of the meniscus, creating an interference pattern in the fluid.

Since the mixing is caused by the interference pattern from the meniscus of the fluid, the mixing is plate format independent, working in 24, 96, 384, 1536, and 3456-well SBS plates.

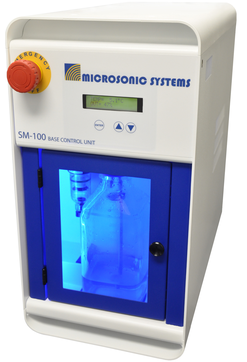
The HENDRIX SM100

The **Hendrix** programmable ultrasonic fluid processor is composed of two units – the Fluid Processor Unit (FPU) and the Base Control Unit (BCU). The micromixer array is built into the small form factor FPU.

The FPU is designed to utilize a minimal amount of the deck space in an automation enclosure. The BCU is designed to reside under the deck of the enclosure.

Fig 6.

Fluid Processor Unit



Base Control Unit

Results

It is easiest to visualize mixing, solubilization, and bead movement with high resolution video. We show here three time-lapse photograph series extracted from a digital video.

Mixing Efficacy

Figure 7 shows time-lapse photographs of dye mixing in water in a 384-well microplate filled with various volumes (a) before mixing, (b) after 5 seconds of mixing, and (c) after 10 seconds of mixing.

Since the mixing is effected from the surface of the meniscus, the mixing is less sensitive to fluid height than acoustic technologies not based on interference.

Mixing with the HENDRIX SM100 is cell-tolerant, affecting neither cell viability nor morphology.

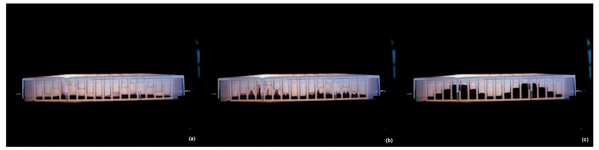


Fig 7. Shows mixing of dye in water. (a) 0 sec. (b) 5 sec. (c) 10 sec.

Compound Solubilization Efficacy

Figure 8 shows time-lapse photographs of the solubilization of granular sugar in water in a 384-well microplate (a) before mixing, (b) after 5 seconds of mixing, and (c) after 30 seconds of mixing where disolution is complete.

Vigorous mixing of compound crystals and ultrasonic vibration of the crystal latice structure quickly desolve crystalized compounds.

The HENDRIX SM100 has been shown to increase the compound solubility of typical inorganic molecules used in compound libraries.

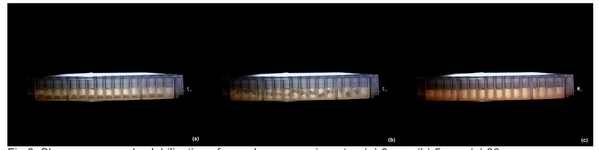


Fig 8. Shows compound solubilization of granular sucrose in water. (a) 0 sec. (b) 5 sec. (c) 30 sec.

Bead and Cell Resuspension

Figure 9 shows time-lapse photographs of the mixing and resuspension of ferro-magnetic beads in a 384-well microplate (a) before mixing, (b) after 5 seconds of mixing, and (c) after 10 seconds of mixing.

Vigorous mixing is required in most biochemical applications that use beads. The inability to mix efficiently in smaller well sizes is limiting their use in higher density plate formats.

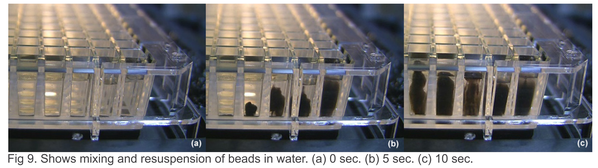


Fig 9. Shows mixing and resuspension of beads in water. (a) 0 sec. (b) 5 sec. (c) 10 sec.

Conclusion

Ultrasonic mixing using our Lateral Ultrasonic Thrust technology enables the move to higher density plates and provides the cost per well savings of assay miniaturization without sacrificing data integrity or assay reliability.

High-speed, high-power, isothermal compound solubilization is now possible for the first time in the microplate format.

Ultrasonic, isothermal thawing now provides a rapid, automated way to transition from frozen storage to an automated system.

The HENDRIX fluid processor is able to keep cells, beads, and particles in suspension neither damaging the beads or particles nor affecting the viability or morphology of cells.

LUT technology has enabled the creation of the HENDRIX SM100 that can perform all of your compound solution mixing, solubilizing, thawing, and resuspension on the same device, at the same time.

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