Control Microplate Dynamics with Covaris AFA Mixing and Dissolution

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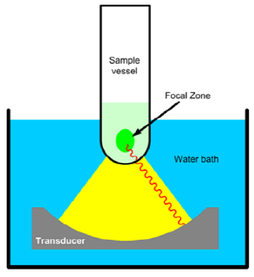
ABSTRACT

Evaluation of a novel ultrasonic line transducer apparatus (L8) developed by Covaris, Inc., which utilizes a non-contact, isothermal Adaptive Focused Acoustics (AFA) process, indicates that low levels of high frequency acoustic energy quickly achieves an advanced state of homogeneity in low volume well plates when compared to agitation methods and mixing by diffusion. In both 96 and 384 well plates, p-Nitrophenol dye was mixed in solution or in dried form with a solution of Sodium Hydroxide. The level of mixing was determined by reading absorbance per well and calculating %CV across the well plate. A single 30 second AFA treatment of the entire plate resulted in both a stable "end-point" %CV values and an improvement in %CV values over traditional mixing processes of agitation or diffusion.

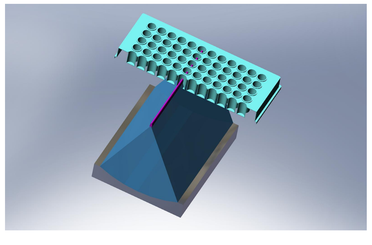
INTRODUCTION

In both High Throughput Screening (HTS) and Compound Management, the working assumption is that solutions are homogeneous as both are negatively impacted by incomplete compound dissolution or precipitation, the latter occurring during storage or after addition of an aqueous buffer. Direct processes to address this concern are often performed by vortexing, sonication, or centrifugation, however, these operate at a macroscopic, bulk fluid level which limits the active control over and the efficiency of the process. Typically the macro-scale process is followed by simple diffusion. This is not controlled in larger volumes, but is especially problematic as the sample volume is reduced. For example, in a 1536 well plate there may be only 4µl of fluid. This small mass is not affected by bulk fluid "mixing" processes that are effective in larger volumes, such as 400µl.



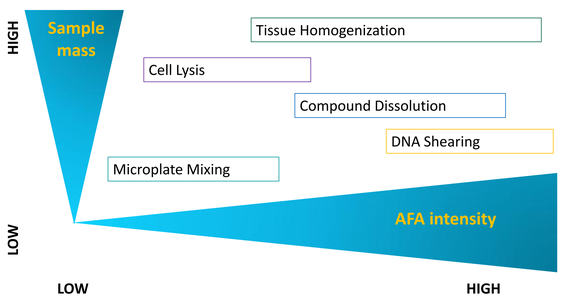
Adaptive Focused Acoustics (AFA) evolved from the highly developed Lithotripsy kidney stone treatment and ultrasound imaging industries. AFA, a patented technology from Covaris, Inc., works by transmitting high frequency acoustic waves from a concave transducer into a sample vessel or well, using water as an acoustic couplant. Acoustic energy is focused into a small, well defined zone within the vessel to create rapid and very complete mixing.

In the Covaris L8 instrument, this technology has been adapted, using a cylindrically shaped transducer, to create a line of focused acoustic energy able to process wells in parallel, in support of a better assay quality and faster processing in a wide variety of High Throughput applications. Typically, a plate is processed in less than 30 seconds.



As shown above, the acoustic focus is a linear zone within an entire column. The Covaris acoustic transducer operates at 850 KHz with a wavelength of ~1 mm. Unlike conventional sonic technology, which employs wavelengths of ~100 mm. The L8 allows acoustic energy to be precisely focused into low volume applications such as (but not limited to) 96, 384, and 1536 format plates in a non-contact, isothermal mode.

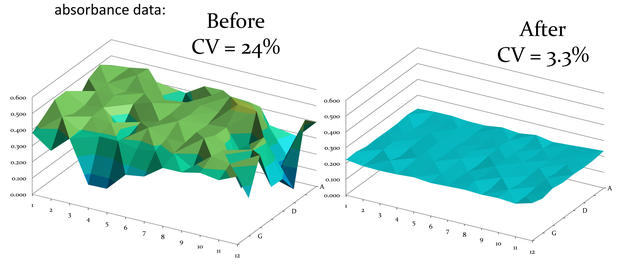
The AFA Spectrum



As with all Covaris AFA instruments, the broad dynamic range of the technology allows the L8 to perform many steps in bioassay and compound screening applications, from rapid compound dissolution, tissue homogenization, DNA shearing, and cell lysis. The applications presented in the schematic represent only a small part of the AFA spectrum of applications. For example, the AFA intensity required for homogenization of hepatic tissue is a fraction of the energy required for a more fibrous tissue such as skeletal muscle. The inherent controlled energy transfer enables precise, accurate protocols to be developed. The process is also scalable across different volumes (or masses). In the L8, acoustic energy is focused to a continuous line, applied to all samples in a column and intermediate, contiguous plate material. Consequently, the maximum energy density available to each well is less than that available from traditional Covaris point-focus systems (e.g., S2, E210, C2000). This limits the upper mass/volume of each sample. For example, while it would be difficult to employ a line focus system to mix 96 wells of 2000µl fluid each, the same task is accomplished easily by AFA systems employing a single point focus, indexing from well to well.

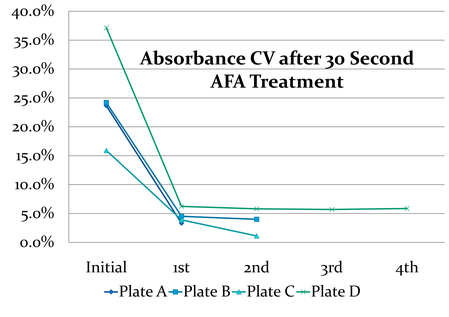
RESULTS – 96 well format

In the following 96 well experiment, 5µl of 9.6 nM p-nitrophenol was dispensed into 100µl of 0.1 N NaOH stock buffer. Absorbance, read soon after dispensing, showed high variance and incomplete mixing. Following a single 30 second Covaris AFA treatment, mixing homogeneity was strongly promoted, as illustrated by the absorbance data:



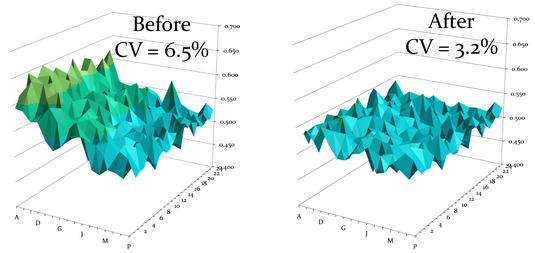
Experimental assistance provided by CyBio

Similar results were seen across a variety of volumes and low-power (under 20 Watts) acoustic treatments in 96 well plates. Repeated treatments demonstrated that end-of-process was typically reached in one 30 second AFA treatment:



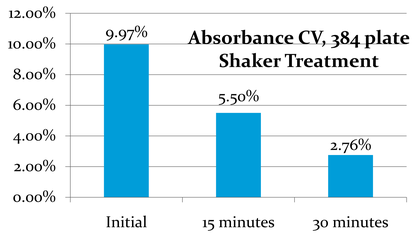
RESULTS – 384 well format

Mixing improvements were also observed in 384 well plates. In the following example, 1µl of 9.6 nM p-nitrophenol was dispensed into 40µl 0.1 N NaOH and absorbance measured prior to the Covaris treatment. Absorbance was again measured after a 30 second Covaris treatment, with resulting CV approximately half the pre-treatment value:

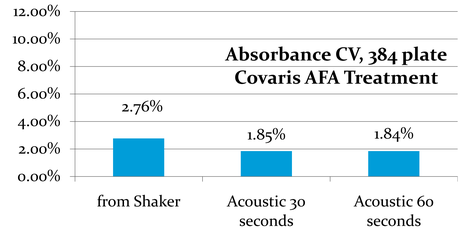


Experimental assistance provided by CyBio

In the following example, 2µl of 9.6 nM p-nitrophenol were placed in each well of a 384 Corning microplate, then dried. To each well was added 40µl 0.1 N NaOH. Absorbance was then measured for each well. The plate was given two consecutive 15 minute treatments in a Jitterbug micro plate shaker set to an intensity of 5. Absorbance was measured following each treatment and %CV calculated in each case:



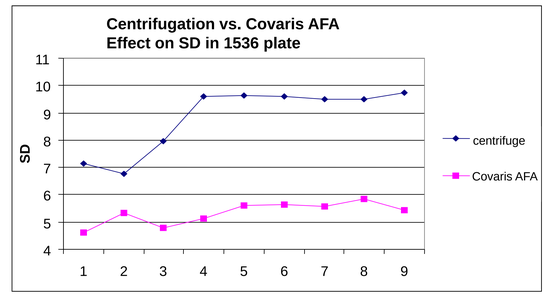
The shaken plate with CV = 2.76% was then given two 30 second AFA treatments in the Covaris L8. The data shows that the Covaris treatment achieved a 49% improvement over shaking results with a single AFA treatment. The additional treatment brought no further improvement, suggesting that mixing end-of-process was achieved within the first 30 second AFA treatment. As this point, CV variation may be due to other factors such as reader, pipette, etc., rather than homogeneity of the solution within the well.

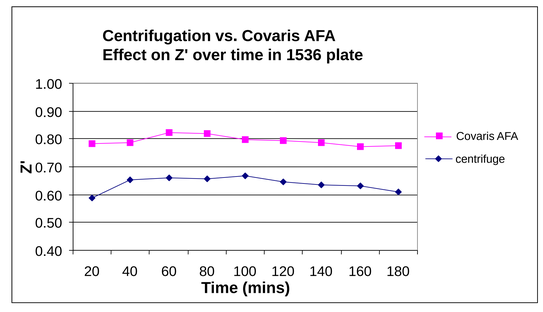


The AFA technology, which has been previously evaluated and validated to be beneficial for controlling and in many instances improving processes such as nucleic acid extraction, compound dissolution, and reagent mixing at a macroscale (100 µl to 10 ml), is now in a format to enable high throughput control of high density microplates. In small volumes (e.g., <10 µl) the traditional physical force mixing processes such as gravitation and bulk fluid flow, are dramatically reduced and their effectiveness diminished. In this state, diffusion is often the resultant dominate "mixing" force. By design, the AFA process is capable of scaling down to the micron level. For example, at the frequencies of the L8 instrument, controlled bubble formation and collapse is on the submicron scale. This is critical for reagent exchange at the domain of the solvent boundary layer. This scaling with the intensity control enables a wide dynamic operating range for the apparatus.

RESULTS – 1536 well format

Mixing experiments for cAMP HTRF detection assay were previously carried out by GSK in 1536 white Greiner plates with 50nl/well of compound and a total assay volume of 8µl. The assay protocol included 4 x 2µl reagent additions using Synquad and Cybiwell, with centrifugation step after each addition. The final centrifugation step was replaced with 800 kHz Covaris acoustic line mixing, making a single pass down the 1536 microtitre plate. Data strongly suggests superior Standard Deviation and Z' results from AFA when compared to standard centrifugation.





CONCLUSIONS

The data shows an overall improvement in mixing and assay performance in terms of elapsed time, %CV and Z'. In the case of the p-Nitrophenol experiment, %CV was immediately improved by nearly 50% in seconds over a 30 minute traditional shaking treatment. In the case of the cAMP HTRF assay, Z' values are improved by a factor of 0.2 and standard deviations improved by 30% or more. Actively transferring mechanical energy into wells in a non-contact manner may provide both control of sample homogeneity and accelerate diffusion-limited processes.

Additional benefits, particularly in the HTS scenario, are accelerated assay time frames, non-contact mixing, and ease of automation. This is especially important for diffusion-limited processes (such as mass action binding events) which are difficult to actively control on a molecular level. As sample volumes are reduced, the gravitational effects on bulk sample mixing are reduced. New technologies are required to overcome the reduced effectiveness. One benefit from the Covaris AFA technology is the scale of mixing achieved by focused acoustic fields driven at megahertz frequencies

The reduction in both mixing time and the increase in mixing quality both support better assay quality and higher throughput. As the improvement of quality systems continues in analytical biology, the use of novel instruments which actively control currently available protocols and processes will increase. The AFA process may be a benefit for the normalization/elimination of diffusion effects for both assay development and High Throughput Screening.