Effects of Wellplate Properties on the Quality of Acoustic Dispensing

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Abstract

Understanding how wellplate properties affect acoustic dispensing is important when selecting appropriate wellplates for an assay or protocol. A collection of simple experiments demonstrates how variations of different plate parameters contribute to the overall quality of the dispense. Properties considered in this study are:

- Diameter of the well
- Bottom thickness of the wellplate
- Surface treatment of the wellplate

Changes in these parameters are measured as a change in the required energy to dispense or a change in the focus position of the acoustic transducer while generating droplets.

Hypothesis

In acoustic dispensing, the source wellplate is directly in the path of the acoustic signal. Properties of the wellplate will affect the acoustic energy that reaches the solution's surface.

The diameter of the well affects the calibration for a specific drop volume. However, the exact nature of this effect is not well understood.

Similarly, the thickness of the bottom of the wellplate is known to have an effect on the drop volume calibration. However, the use of several different plates with different bottom thicknesses on the same acoustic dispenser is extremely rare.

Surface treatment of a wellplate changes the behavior of how a solution interacts with the surface. Treating the surface with plasma will effect the shape of the meniscus of a solution in a well, and in turn, effect the performance of acoustic dispensing.

Materials and Methods

All studies were performed using the ATS Acoustic Transfer System from EDC Biosystems. In addition, a collection of wellplates from Aurora, Genomic Systems, BD Falcon and Greiner Bio-One with different properties were used.

Well Diameter

A standard calibration for the ATS is a set of requirements, as a function of liquid depth, needed to generate a desired drop volume. This set of requirements define the impulse, the force times time, needed to generate the drop. Figure 1 is a geometric representation of the acoustic signal generated by the ATS traveling through four superimposed source wellplates with well diameters ranging from ø1.4 mm to ø3.2 mm.

Well Diameter Data

The attenuation data was collected by making the assumption that the impulse power required to generate a drop of a given volume remains constant as a function of the amount of liquid in any given well. For a known speed of sound and a constant distance between the acoustic transducer and the liquid surface, the amount of energy needed to generate a 2nL droplet is determined. The assumption is also made that for very low liquid levels there is little or no attenuation. This implies that at low liquid volumes, required power divided by the actual needed power for a given liquid level, is the attenuation of the signal.

This data was acquired from four different well diameter wellplates and is displayed in Figure 2. The data shows that the assumption that the required power to generate a 2nL droplet for liquid levels less than 1mm is the same regardless of the well diameter. The data also shows that the liquid level where the rapid fall off begins, changes as a function of well diameter, which agrees with the assumption that a larger well will allow more signal to pass through, while a smaller well will begin to attenuate the signal sooner.

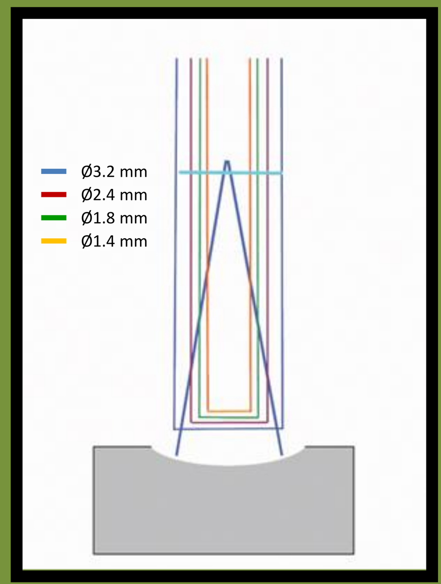

Figure 1. As the well diameter decreases, the acoustic cone is cut off at lower liquid depths.

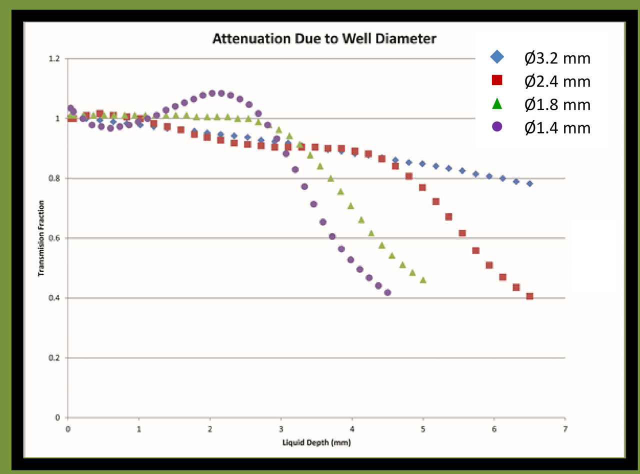

Figure 2. Attenuation of the acoustic pressure as a function of the level of liquid in the well.

ATS Acoustic Transfer System

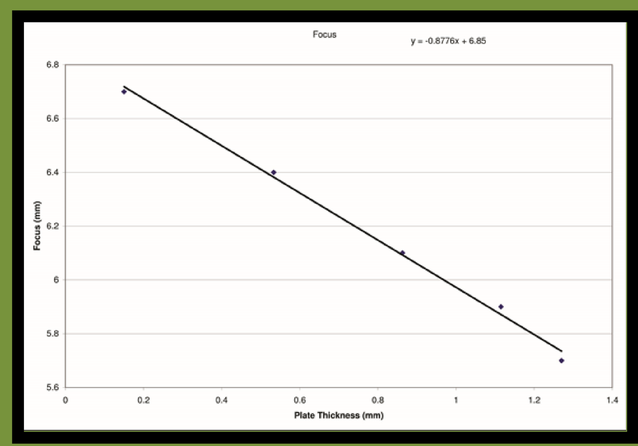

Figure 4. Ideal focus as a function of plate thickness.

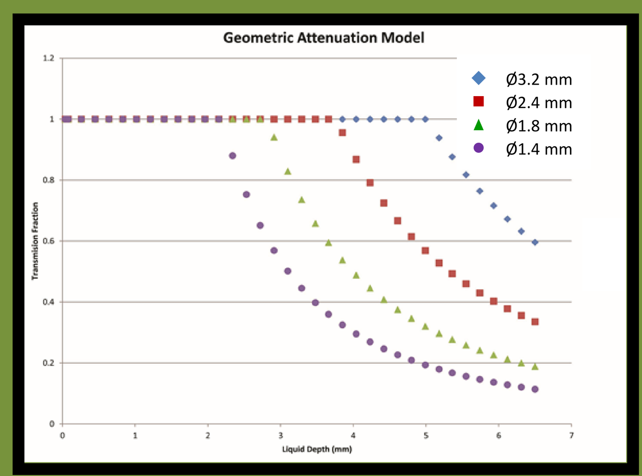

Figure 3. Acoustic energy that makes it into the well is used to generate the droplet.

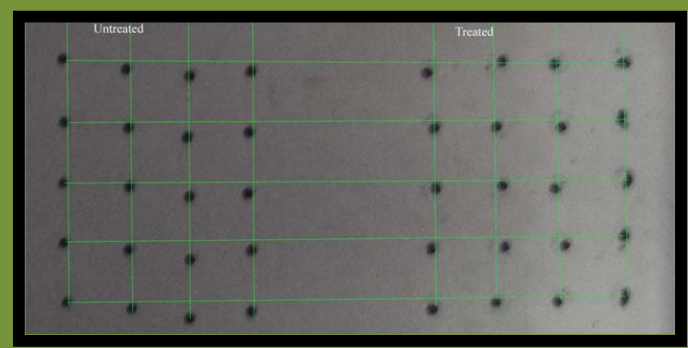

Figure 5. Comparison of 50 nL droplets dispensed from treated (right side) and untreated (left side) wellplates.

Wellplate Bottom Thickness

Five different plates with well bottom thicknesses of 0.15, 0.525, 0.85, 1.15, and 1.25 mm were used to study the effect of plate thickness on acoustic dispensing. The plates are all made from polystyrene, therefore plate material should not affect the results.

Bottom Thickness Data

In the case of well bottom thickness, the method of creating calibration curves and looking at the attenuation yielded only a slight attenuation effect. However, the effect on the thickness, the parameter that changes turns out to be the focus. The proper focus is determined by trial and error testing for the minimum energy required to generate a given drop volume. By using this method, Figure 4 - Ideal Focus, as a function of plate thickness for polystyrene, was generated.

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Surface Treatment of Wellplate

Two plates, one treated and one untreated, were used to study the effect of plasma treatment on acoustic dispensing. The micro patterning of a plastic surface can change a surface from hydrophobic to hydrophilic. This is because the micro pattern is a groove in which water is attracted by capillary action. Once the surface is wetted, the surface contact angle is lowered. For wellplates, this micro patterning may be created by exposing the plastic surface to plasma. The plasma etches tiny grooves in the surface that the water is attracted to.

Surface Treatment Data

The effect of plasma surface treatment on a plate lowers the hydrophobicity of the surface. This action affects the way the meniscus interacts with the well walls. In acoustic dispensing, an acoustic beam is focused on the surface of a liquid and the reflection of that energy causes the surface to rise. As more energy is applied, the surface rises enough so that the surface wraps around a bolus of liquid. Momentum takes over, allowing a droplet to form and be projected out of the surface. It is important to note here that the surface of the liquid pulls back on the droplet as it moves. Most importantly, if the trajectory is not perpendicular to the surface, the droplet will be pulled in the direction of the smallest angle with the surface.

Summary & Analysis

Well Diameter Analysis

In order to verify whether a geometric model for the attenuation adequately describes the phenomenon, a simple ray diagram was generated. Assuming that the distance between the acoustic transducer and the liquid surface is constant, there will be a point where the "acoustic cone" will be attenuated by the edge of the well wall and block that portion of the signal. By calculating the area of the conic section at that point and dividing it by the area of the well bottom, the fraction of transmission signal is calculated. The data for the same four wellplates are shown in Figure 3.

Well Thickness Analysis

The data in Figure 4 suggests that the wellplate bottom acts as an acoustic lens in such a way that it changes the focal length of the acoustic signal. Therefore, the ideal focus needs to be adjusted to compensate for the plate thickness.

Surface Treatment Analysis

Figure 5 shows 50nL dispenses from both a treated and an untreated plate. The green lines represents the intended grid pattern. Each row is dispensed from the same set of wells, therefore one can observe how the same well responds similarly dispense after dispense, until the meniscus realigns itself during the draining process. One can also observe that the meniscus in the treated plate moves around after each dispense more easily than the untreated plate. On average, the treated plate menisci all tend to have the same shape, which is derived from the observation that the dispenses all tend to land closer to the grid centers.

Conclusion

Properties of the source wellplate affect the quality of the acoustic dispense. Three of these properties, well diameter, well bottom thickness, and surface treatment were studied in this experiment. The diameter of the well leads to an attenuation of the acoustic signal. The thickness of the well bottom affects the focus of the acoustic signal. A hydrophilic surface treatment affects the meniscus and improves the placement distribution of the drops. By understanding these properties and their effects, the performance of the ATS acoustic transfer system may be improved.