EFFECT OF FOCAL DISTANCE ON DROP VOLUME IN ACOUSTIC DROP EJECTION

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INTRODUCTION

At EDC Biosystems, acoustic energy is being used to create and eject small droplets of liquid out of microtiter wellplates and onto targets. The target is usually another wellplate or perhaps a glass substrate. These droplets are used in chemical reactions where the volume of the droplet is an important parameter. This being the case, the accuracy and precision of the drop volume needs to be understood. Several parameters effect the volume of the drop, including the total acoustic energy, the amount of liquid in the well, and the focus of the acoustic energy on the surface of the liquid.

METHOD

EDC Biosystems' *True Non-contact Technology*™ uses focused acoustic energy to eject droplets of liquid out of a microtiter wellplate. In order to do this, the amount of liquid in the wellplate is measured by using a sonar measurement technique. A short burst of acoustic energy is generated and the time of the reflection of the pulse off of the surface of the fluid is measured. Based on the speed of sound of the materials that the pulse travels through, the amount of liquid in the well is determined. This parameter can then be used to adjust the acoustic energy and the distance the acoustic transducer is placed away from the liquid surface.

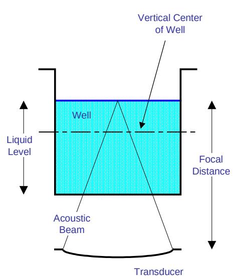


Figure 1

EXPERIMENTATION

WELL DRAINING

A well draining experiment is an experiment where droplets of liquid are produced from the same well. As more drops are ejected from the well, less liquid is left in the well. This type of experiment allows for a large sample of data at various liquid levels. Continuous measurement of the amount of liquid in the well allows for the determination of the amount of liquid ejected from the well on average.

INITIAL INVESTIGATION

A simple draining experiment is to position the transducer so that the nominal focal distance is fixed in the vertical center of the well as shown in Figure 1. Droplets are ejected continuously without changing the vertical position of the transducer or the amount of applied acoustic energy. The droplets land on a glass slide and are photographed. The diameter of the droplet is determined using object measurement software. The actual volume of the droplet is estimated based on this measurement, fluorescence data, and amount of total liquid ejected from the well over the draining experiment. Figure 2 shows the results of this investigation.

The Initial Investigation shows a regular variation in the volume ejected. The maximum volume ejected corresponds to the point when the well is about half full. This is also the liquid level where the surface of the liquid corresponds to the nominal focus of the acoustic beam. It could be argued that the maximum volume is ejected at this point because the acoustic waves are in focus, and therefore the ability to eject droplets is efficient at this point.

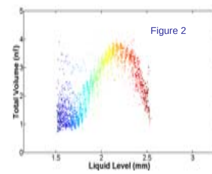
CONSTANT FOCUS

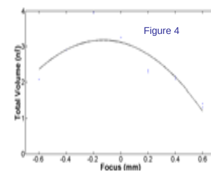
Based on the Initial Investigation, one might hypothesize that in order to keep the efficiency of the drop ejection consistent, the nominal focal distance of the acoustic beam should be held at a constant distance from the surface of the liquid as the well is being drained. The focal position is the distance between the nominal focal distance and the surface of the liquid. Therefore, a second draining experiment maintaining a constant focal position was performed. By keeping the focal position constant, the dependance on the focal distance of the volume ejected is removed.

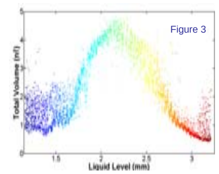
The focus of the acoustic beam is determined to be the point where the area of the acoustic beam on the surface of the liquid is minimized. This also means that the pressure of the acoustic energy per unit area on the surface is maximized. At the focal point, two things happen. First, using the minimum energy needed to create a drop for a given liquid level, one can create the minimum drop volume. This minimum is dependent on the area of the circle of pressure on the liquid surface. Second, the best efficiency for creating a drop occurs.

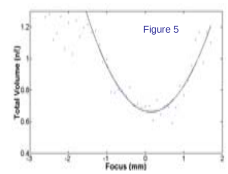
In this second experiment, the position of the acoustic transducer is moved based on the distance of the transducer to the liquid surface in order to maintain a constant focal position. After moving to this position, a constant amount of energy was used to produce a droplet. The droplet volume is estimated using the same technique as described above. The data shows that the volume of liquid ejected is once again dependent on the level of liquid in the well. Figure 3 shows the volume of the droplets as a function of liquid in the well for this experiment.

From this experiment, it is clear that the amount of liquid in the well has an influence on the volume of the drop ejected, even when the energy and focal distance are maintained.









ATS-100

Acoustic Transfer System

RESULTS

EFFECTS OF DIFFERENT FOCUS VALUES

The constant focus draining experiment can be performed with different values of focal distance. The drop volume is then measured and plotted as a function of liquid in the well as demonstrated above. By measuring the volume over the range of liquid levels for several values of focus, the behavior of the drop ejection process may be compared for different focus values. Several different fixed focus values can be used to determine whether the behavior is due to the particular lens position chosen or a property of the well and liquid level itself.

One way to compare the effect of focus on acoustic ejection of drops, is to select a particular level of liquid in a well and take an average of the measured drop volume for this liquid level. Calculating this average for a series of different focus points and plotting those points, shows the effect of different focal points on the drop volume ejected.

This data is then used to determine the effect of focus at particular liquid levels. Figure 4 shows the average drop volume for drops ejected from a well filled between 1.9 and 2.1 mm deep as a function of focus. This demonstrates that the efficiency of drop ejection increases as the acoustic beam comes into focus.

MINIMUM DROP SIZE

The final effect of focus is related to the area of the acoustic beam on the surface. Not only does the drop volume increase because of the improved efficiency as the beam comes into focus, but the actual area of the acoustic pressure becomes smaller as the beam is focused. Therefore, the minimum drop size ejected can be determined as a function of focus.

Selecting a focus and reducing the energy used to eject a drop until it no longer spots yields the minimum drop size. This can be done for various focus settings until the dependence on focus can be seen. Of course, this experiment is repeated for various liquid levels because of the variation that we have already documented. Figure 5 shows the minimum drop size as a function of focus.

CONCLUSION

Focus of the acoustic beam has an effect on the efficiency and the minimum drop volume parameters in acoustic drop ejection. Control of the focus and the energy used to eject a drop is critical to control of the drop volume created. By measuring the amount of liquid in the well one is able to determine the focus and energy required to eject the expected drop volume.