Utilizing High Speed Photography to Optimize Low Volume Dispensing Conditions

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Overview

Objective: Improving positional dispensing precision of low volume (nanoliter) drops and understanding the factors that affect them. These same parameters can be investigated to reduce deleterious effects on dispensing performance such as deflected streams, satellite formation, secondary pulses and drop deformation.

Why: Many applications, such as those in clinical diagnostics and low volume crystallography require not only precise volumetric dispensing, but also precise drop positioning. These applications are also less tolerant of satellite formation and other phenomenon that cause reagent to be located outside the required area of interest.

How: Using a high-speed camera to record the dispensing stream from a Nanodrop automated liquid handler.

Variables that are investigated:

- Pressure of dispense (backing pressure)
- Airgap size
- Reagent chemistry

Materials used:

- Liquid handler: Nanodrop ExtY
- Reagents: 30% glycerol, 100% DMSO, tris buffered saline solution pH 8, YOx beads in suspension
- Camera: MotionPro® PCI 8000S motion analysis video camera from Redlake Imaging

Results: Improved plate precision and dispensing profile for several diverse reagent types.

Materials and Methods

The high-speed camera used for this study was a Redlake Imaging PCI 8000S, which would take continuous video at a rate of 2000 frames/s (magnified at approx. 200x) and pass this to a video capture board on a PC, generating a video file in Windows AVI format. Captured videos can be analyzed frame by frame. The dispensing tip was located .94 cm from the surface of a glass slide. The camera was focused on the end of a single tip, at a distance of about 2 feet, with sufficient depth of frame to show the tip end and slide surface simultaneously. The setup is shown in Figure 1 (at right).

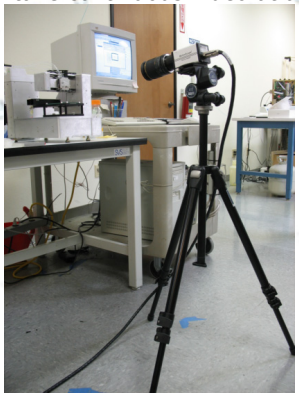

Fig. 1 Redlake Imaging Camera and Innovadyne Nanodrop ExtY

The following parameters were varied:

- **Pulse Width**: the open time of the micro-solenoid valve. The longer the open time, the larger the dispensing volume.
- **Pressure**: the helium pressure applied to the system fluid reservoir in order to force the liquid out the tips through the solenoid valves. The greater the backing pressure, the larger the volume dispensed within a given solenoid open time. A higher pressure allows cleaner cleavage of reagent from the dispensing tips, but could cause splashing or pressure backlash from the tips if it is too high.
- **Airgap Size**: the volume of aspirated air that separates a sample from the system fluid (deionized water). A larger airgap will decrease the likelihood of a sample being diluted with system fluid, but may increase the absorption of the applied pressure, resulting in slower ejection velocities and more pressure bounce.

Pulse widths were chosen such that the actual dispense volume was approximately the same. In this case the dispense volume was approximately 250 nL, which corresponded to pulse widths of 7000 us, 5000 us, 4000 us and 3000 us for 8, 12, 16, and 20 psi, respectively.

An 8-tip Nanodrop ExtY instrument from Innovadyne Technologies, Inc. (see Fig. 2) was used as the automated liquid handler both for high-speed photography of dispenses and for plate precision testing.

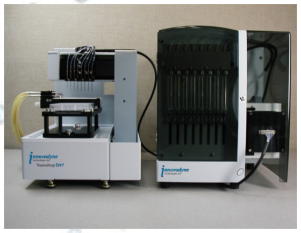

Fig. 2 Nanodrop ExtY

Liquid velocity was determined by counting the number of pixels that the leading or trailing edge of the stream moved within a frame. Knowing the distance per pixel and the number of frames per second, we were able to come up with velocity measurements for 2 to 5 points within the stream using the following equation:

Velocity = #pixels * distance per pixel * frames per second

These velocities were averaged to determine the overall velocity of the liquid stream. The effects of gravity on the velocity of the stream are assumed to be negligible within the time frames used in this study.

Results

Liquid Velocity and Profile

Using the high-speed camera, we are able to determine the velocity of the ejected drops. As we would expect, the calculated velocities are generally proportional to applied backing pressure. This can be seen in Fig 4 to be true for both the 30% glycerol and tris buffer reagents investigated, however, for DMSO the velocity does not seem to change with pressure.

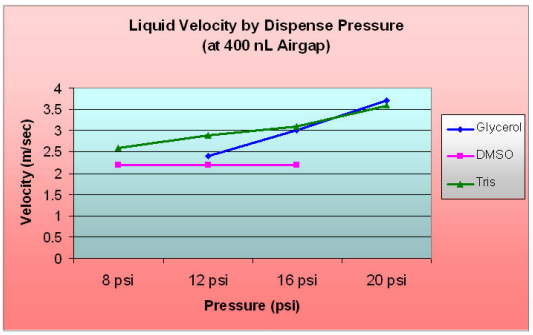

Fig 4 - Liquid Velocity by Dispense Pressure

Another observation of interest was that the ejection velocity decreased with an increase in airgap size. This effect, which is applicable to all reagents studied, can be seen in Fig. 5. While this effect is not large, it suggests that there are several ways with which one can optimize the dispense velocity, and presumably also the dispense profile.

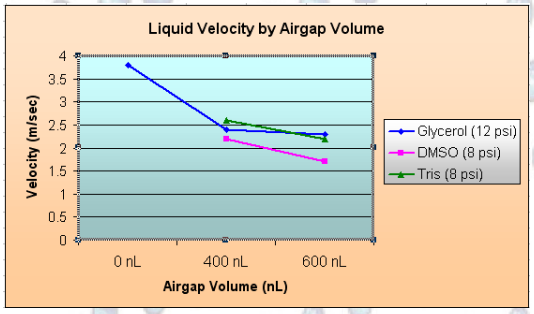

Fig 5 - Liquid Velocity by Airgap Volume

Secondary drops are seen in several of the videos presented in this work. Conditions were chosen such that a wide range of dispensing profiles could be observed. Secondary drops are occasionally formed after the primary stream of liquid has been dispensed. While most often these drops simply join the droplet formed by the primary stream, on occasion these droplets have bounced off the primary drop or have emerged from the dispensing tip at a slower velocity such that they are able to be deflected from the vertical stream and land to the side of the main droplet. It is these secondary drops that are likely the cause for much satellite dispensing that can be observed in low volume dispensing.

Typically, these satellites are not an issue in standard microtiter plates (all tip Cvs were below 4% for all dispensing conditions studied here), however, as dispensing densities get larger and other applications arise, they can be far less forgiving of positional errors. These satellites then become more detrimental to the dispensing performance of the instrument. Having the flexibility to vary dispensing parameters in order to optimize the dispensing profile of the liquid is imperative. Generally, secondary dispenses are seen both when the drop ejection velocity is too low and when it is too high. It is presumed that they are caused by some form of backlash pressure that occurs after the solenoid has closed, but it is not clear why this happens at both pressure extremes and not in the middle pressure range of dispensing.

30% Glycerol

Due to the higher viscosity of 30% glycerol (2.4 cP) relative to water (1.0 cP), it would be expected that a higher backing pressure would be required to dispense this solution. Indeed, we can see that while 12 psi dispensing of 30% glycerol into a 384-well plate results in acceptable tip Cvs (2.9%), the high speed photography shows that the dispense stream produces secondary drops (Figure 6). It can also be seen that increasing the aspirated airgap further contributes to the presence of secondary drops. One can reduce the presence of these secondary drops by either eliminating the airgap or increasing the pressure (in this case to 16 psi).

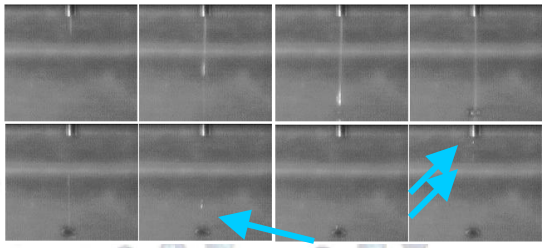

Fig 6 - Video Excerpts, Suboptimal Dispense of 30% Glycerol (12 psi 400 nL Air Gap)

The following chart shows conditions under which secondary drops are present in the high-speed videos.

Table 1 - Satellite Presence/Absence for 30% Glycerol Under Varied Pressure/Airgap Combinations

	12 psi	16 psi	20 psi
No airgap	absent	-	absent
400 nL	present	absent	absent
600 nL	present	-	present

DMSO

Upon first thought, one might think that DMSO would also dispense better using higher dispense pressures, as the viscosity of DMSO is similar to that of 30% glycerol (2.14 cP). However, we can see from the high-speed videos that the best dispense profiles actually occur at the lower pressures, such as 8 psi, as opposed to the higher pressures of 12 or 16 psi. As mentioned earlier, the ejection velocity of this reagent does not seem to increase as the backing pressure is increased, which suggests that the increased backing pressure is being absorbed through other means. We also see the formation of secondary drops as we move to higher pressures. Again, a larger airgap is somewhat detrimental to the dispense profile and we see secondary drops forming as the airgap is increased. This certainly implies that the dispensing profile is dependant on more than just viscosity, but also potentially on characteristics such as surface tension and hygroscopic tendencies.

Tris Buffer

As a simpler reagent in comparison to DMSO and 30% glycerol, a 0.05M tris buffer solution behaves in a much more predictable and tolerant manner, resulting in excellent dispense profiles up until 20 psi, where secondary drops can be formed. Increasing airgap sizes are expected to effect dispense profiles for this reagent as well, however, the conditions used in this study did not reach the point where this is noticeable in the videos.

Other Solutions

While the reagents investigated in this study were limited to the three discussed above, other reagents such as ethanol and yttrium oxide bead suspensions have been optimized as well. Fig. 9 shows the dispensing profile of 250 nL of a 80 mg/mL YOx bead suspension.

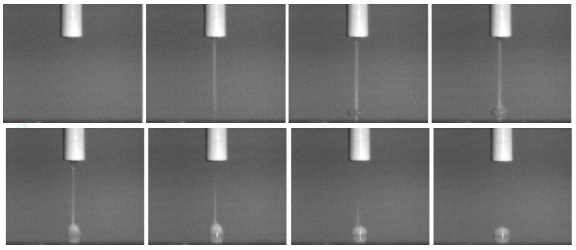

Fig. 9 - Non-contact dispense of YOx beads

Conclusions

We have been able to demonstrate the following:

- High speed photography can be utilized in order to optimize the dispensing profile of a variety of reagents.
- Good precision data in a microtiter plate format is not always an indication that dispensing profiles are good.
- DMSO should be thought of differently than most reagents, as its dispensing velocity does not appear to increase as backing pressure is increased.
- Often dispensing conditions may be optimized using several different parameters including changing pressure and airgap.