Technical Note: 2102

Key parameters for a good liquid transfer to Lazwell plates

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Keywords: Lazwell, LDTD, liquid transfer

Introduction

When using the LDTD technology, adequate plate spotting is essential to attain an optimal performance. There are key parameters that need to be optimized to obtain an efficient liquid transfer into the self centering portion of the well.

Even though this step is quite important to get the most out of the instrument, it is easy for the liquid to get out of the center cup of the well, particularly when using a liquid handler system. Understanding the physics involved in the dispensing of the liquid enables us to find solutions to achieve an adequate liquid transfer.

Basic considerations

Figure 1 shows the optimal position of the liquid into the centering cup of the well. There are several conditions to consider to place the drop in this position such as the liquid composition, the pipette tip material and shape, the temperature, etc.

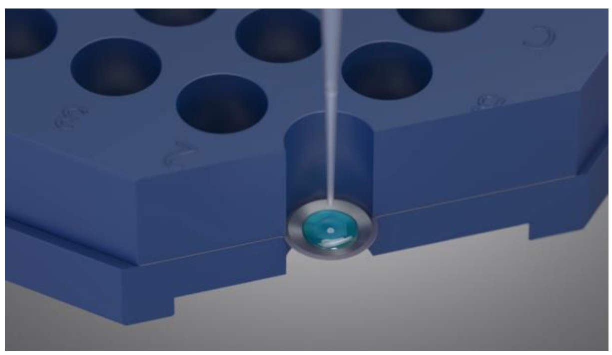

Figure 1 - Optimal liquid transfer positioning

The most important parameter to consider is the contact angle (function of the surface tension and surface energy) of the liquid in conjunction with the metal bottom of the well. The contact angle with the surface determines the spread diameter into the centering cup. Figure 2 shows the different possibilities from high (A) to low (S) contact angles. As an example, trying to transfer pure methanol into a well will result in a spill out of the centering cup because of the low contact angle (θ_C) with the metallic surface. The combination of a low surface tension (air-liquid) and a high surface energy (liquid-solid) results in a low contact angle. As a comparison, the surface tension of methanol at room temperature is about 22.6 dyn/cm compared to water at 71.96 dyn/cm.

Figure 2 – Drop shape on a surface for different contact angles (θ_C)

Surface tension is dependent on temperature, and generally, that surface tension decreases when the temperature increases. Therefore, the temperature should be kept as stable as possible during the transfer of the liquid into the plate. There is also a dynamic behavior when the liquid is moving quickly over a surface: the contact angle can be altered from its value at rest. The discrepancies between static and dynamic contact angles are closely proportional to the capillary number, based on the fluid viscous drag force.

Practical considerations

LDTD technology is used in multiple applications with several matrices and liquid types. The user has to determine the best approach to get the sample transferred properly into the Lazwell plate.

Liquid contact angle

- When using an alcohol solvent such as methanol, keep the aqueous content of the liquid high to increase the surface tension. The resulting contact angle will favor the centering of the drop. 25% aqueous content should be considered as the minimum value.
- Work at room temperature, avoid warm liquids or a warm LazWell plate.
- Use slow dispense speeds: dynamic contact angle is reduced as the speed increases. A speed of less than 5 µL/sec is recommended unless working with a special case (ex: dispensing hexane).
- Use an appropriate pipette tip material: liquids with a low surface tension can spread more or less on the exterior of the pipette tips as a function of the wettability of the surface. Figure 3a shows a 100% methanol dispense from a clear polypropylene tip. The drop detachs from the tip at room temperature. On Figure 3b, the drop of methanol wets the black conductive tip (polypropylene with a carbone additive) and crawls up the exterior of the tip. The addition of an aqueous solution helps the dispense of the liquid from the black conductive tip (Figure 3c).

Figure 3 - a) Clear Polypropylene tip with 100% MeOH. b) Black conductive tip with 100% MeOH. c) Black conductive tip with 70% MeOH and 30% water.

- For liquids that are immiscible with water (hexane, MTBE, ethyl acetate), the material of the tip is crucial to avoid loss on the exterior of the tip and to improve the transfer efficiency. As there is no direct possibility of increasing the contact angle, the volume transferred must not exceed 6 µL so as to limit the excess of liquid that would be lost by slipping out of the centering cup. Dispense speed will be an important parameter to tune as a higher dispense speed keeps the drop on the point of the tip but ejects the liquid out of the well centering cup. The expected speed should range between 5-10 µL/sec.

Liquid handler dispense

- The LDTD technology is often used in high throughput analysis which can generate a huge number of samples. The use of a liquid handler is an important part of the workflow to enable such a throughput. An efficient liquid transfer is attainable when paying attention to these key parameters:
    - **Tip material:** Clear high density polypropylene is the material of choice. Low surface energy enables a large contact angle with most liquids. Black conductive polypropylene tips have a higher surface energy and a wettability with which some liquids are not compatible and a higher aqueous content is required to achieve a good transfer.

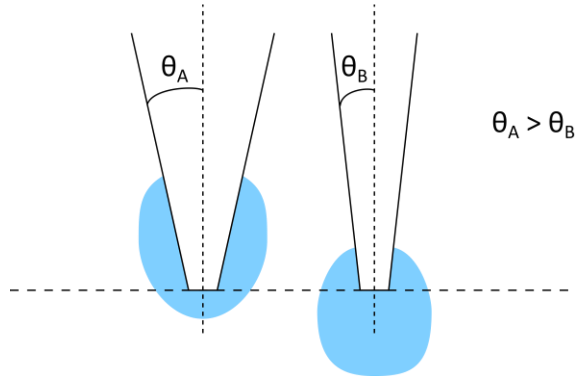

Figure 4 - Drop shapes on tips with different angles

- **Tip shape:** Choose thin and straight pointed tips. A higher angle (θ_A) compared to a vertical line increases the spread of the liquid on the exterior of the tip. Conversely, a lower angle (θ_B) between the surface tip and a vertical line will limit the spread of the liquid on the exterior of the tip (Figure 4).
- **Fixed tip:** Users must adjust the aqueous level of the sample when possible to get a higher contact angle with the coating of the tip.
- **Dispense speed:** A low dispense speed keeps the contact angle near its maximum value. The speed must be lower than 5 µL/sec unless it is a special case. A faster dispense may be needed if the surface energy of the tip material favors the spread of the liquid on the outer surface of the tip. Increasing the speed helps to eject the drop from the tip using the momentum of the liquid. This is a trade-off with the liquid's spread due to the dynamic reduction of the contact angle on the well surface.
- **Dispense position:** A good starting point is 1 mm above the surface of the bottom of the well. The drop size depends on the volume transferred but generally will be 2-3 mm in diameter. To ensure a proper transfer, a bottom tip touch function can be used.

Example 1

Effect of the ratio Methanol/Water

Figure 5A shows an example of the behavior of the liquid for various ratios of a Methanol/Water mix with red dye. A volume of 8 µL is deposited into each well with caution to keep the drop into the centering cup.

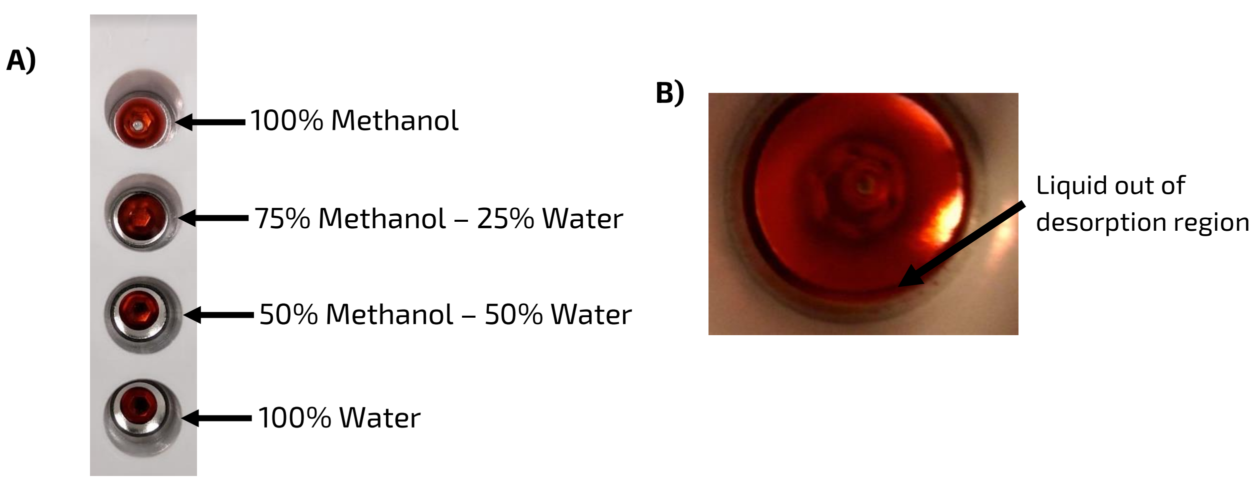

Figure 5 – A) Liquid dispersion into the centering cup. B) 8 µL deposit of 100% Methanol

A closer look at the 100% Methanol is presented in Figure 5B. A portion of the liquid escapes the centering cup as small drops are visible outside of the desorption region. This will increase variability in the analysis. Using a smaller volume with reduce this effect. Other liquids with low surface tension like Hexane, MTBE and Ethyl Acetate will behave similarly.

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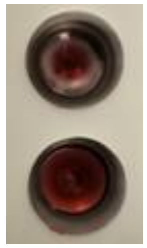
Example 2

Splash out

When the liquid breaks the outer edge barrier of the centering cup, a large portion of the transferred volume escapes out of the desorption region. **This provokes a major method failure** as most of the sample is not analyzed. Figure 6 shows the difference between 8 µL of 100% Methanol that breaks the barrier with only a few µL remaining into the desorption region compared to the same volume contained within the limit. The user must note that with such low surface tension liquids, splash can occur with tiny variations such as vibrations, temperature modification, liquid contaminants, etc. Corrective actions are required to ensure an adequate liquid deposition.



8 µL of 100% Methanol splashed out of desorption region

8 µL of 100% Methanol within centering cup edges

Figure 6 - Example of liquid splashing out of the edge of the centering cup

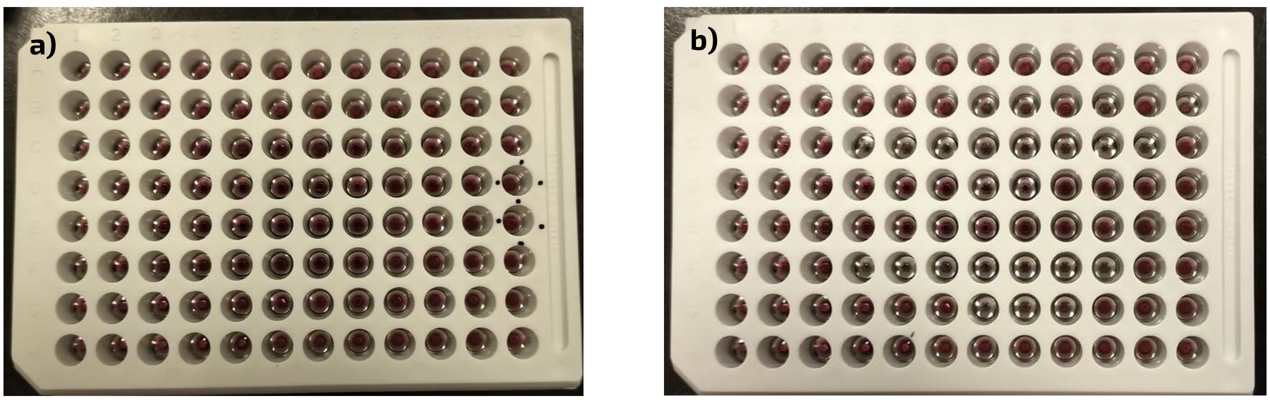


Figure 7 - Example of sample loss by slightly bumping the plate

Common liquid transfer errors occur during the manipulation of the plate after the liquid dispense. In the case where a liquid with a low surface tension is used, the user must leave the plate untouched to let a portion of the solvent evaporate before it is moved. Figure 7a shows the transfer of 7.5 µL of a mixture of IPA-MeCN-Water with a ratio of 75:20:5 in all wells. Figure 7b present the same liquid transfer but the plate was bumped slightly. A few wells spilled out of the centering cup. The remaining liquid volume in those wells is highly variable and represent a small fraction of the original volume.

Conclusion

Managing to have the highest surface tension possible, the best pipette tip material and a tuned dispense speed with bottom touch tip function is the key for a complete liquid transfer process into the Lazwell plate. Working with key parameters in their optimal zone ensures that the user obtains an efficient plate spotting into the self centering portion of the well which produces optimal LDTD data quality.

If you encounter difficulties with the liquid transfer into the Lazwell plate, don't hesitate to contact Phytronix to get support at **luxon.support@phytronix.com**.

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