VistaLab™ TECHNOLOGIES

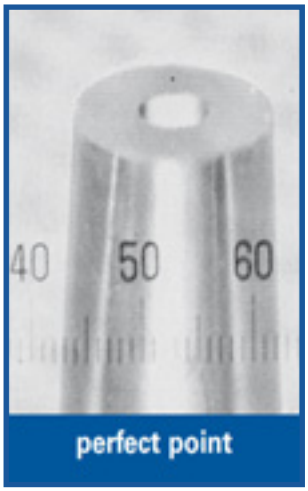
**TECHNICAL NOTE**

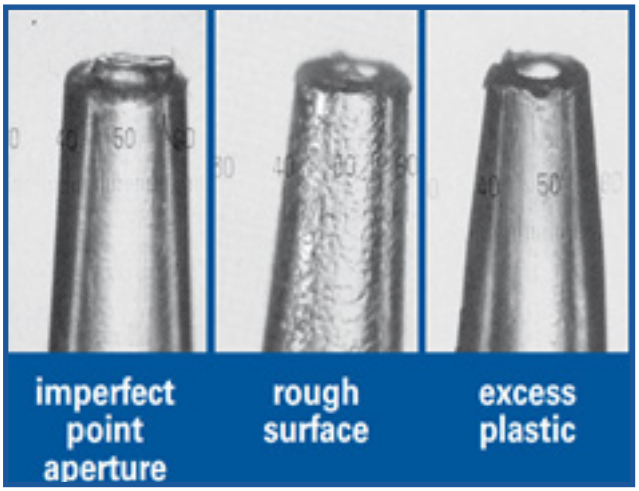
# Micro-Volume (<0.2 µL) Pipetting: Techniques and Tips

Any experienced pipetter knows that when pipetting, the smaller the dispense volume, the harder it is to achieve accurate and consistent results. Pipetting volumes below 2.0 µL can be frustratingly difficult; the smallest drop left behind, the slightest movement, or the quality of the individual tip can cause poor results. A pipette that fails calibration for one technician, may pass for another. Not all variables can be easily controlled, but here are some techniques and tips that can help improve performance:

## 1. Not every tip is created equally:

No two snowflakes are identical. The same goes for pipette tips. Even the highest quality tips can have the slightest imperfections or deviations. The effect is usually negligible when working at higher volumes, but volumes below 2.0 µL are extremely sensitive to these imperfections. When applying a new tip to your pipette, always inspect it for excess plastic ("flash") and other imperfections at the outlet orifice. Also, treated tips, such as Low Retention tips, can adversely affect performance because of the hydrophobic forces acting on the small volumes of liquid.




## 2. Consistent technique for consistent results:

Technique is always crucial to obtaining consistently reliable results especially when working at micro volumes.

Aspirating:

- Keep tip completely vertical.
- Depress plunger to first stop,
- Immerse tip 3mm into center of liquid.
- **Slowly** release the plunger, **pause** for 2-3 seconds, then **slowly** remove tip from center of liquid.
- If any liquid remains on outside of tip, carefully wipe off, making sure to not 'wick' any liquid from inside of tip.

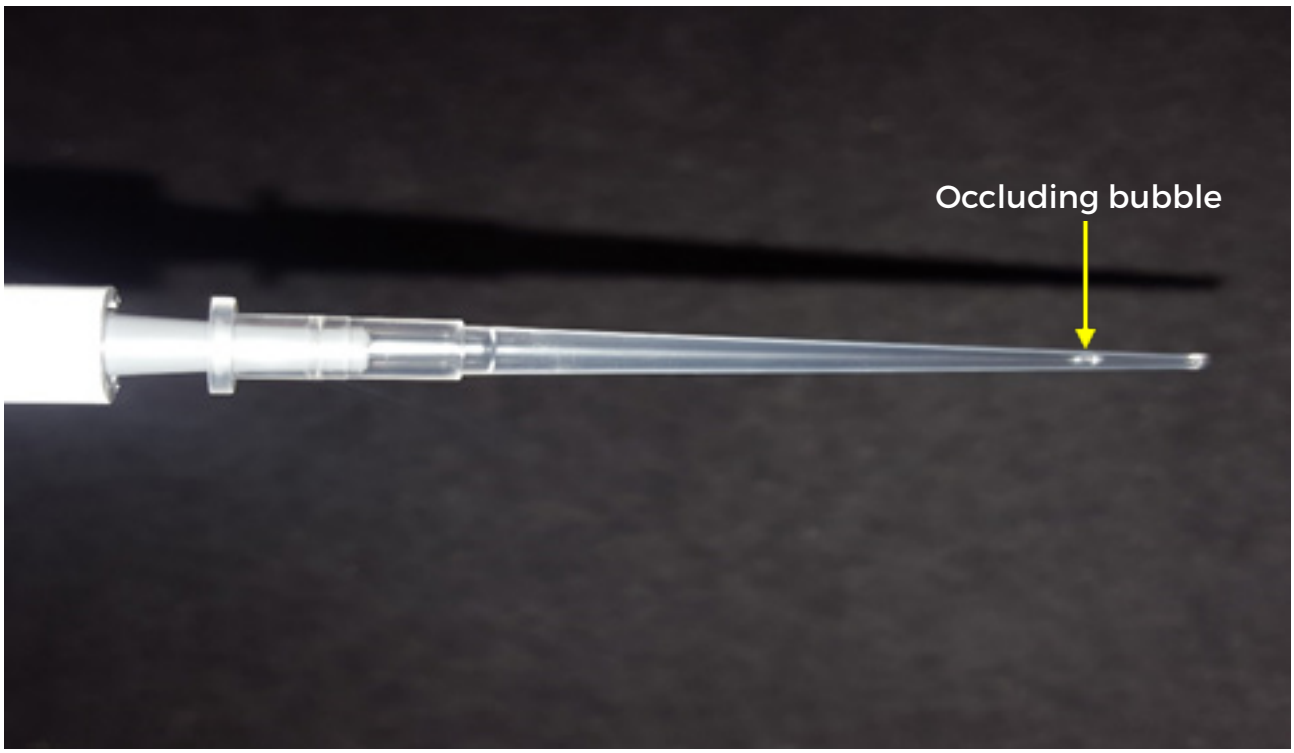
Dispensing:

- Angle tip approx. 45° and touch to side wall of receiving vessel or just above the surface of the liquid.
- Press the plunger through to the second stop (blowout) and **hold.**
- Touch off on vessel wall and drag the tip up along the wall to remove any liquid remaining at tip*.

*Keep plunger completely depressed until all liquid is removed from tip. Be careful not to drag tip through previous aliquots, as liquid may adhere to the outside of the tip and affect the measurement.

3. **Beware of occluding films/ bubbles:**

When liquid drops are not properly wiped or touched off, they can be inadvertently re-aspirated into the tip. If the drop is large enough, the surface tension can cause a liquid film or bubble that can restrict the air flow and consequently affect the next aspiration and dispense cycle.




**Solution:**
Dispense into receiving vessel against the vessel wall. Always keep the plunger fully depressed through the second stop (blowout) after dispensing and thoroughly wipe the tip along the vessel wall. When removing the tip from the receiving vessel, **keep plunger fully depressed**, inspect tip for residual liquid, and continue to touch off on the receiving vessel wall until all liquid is removed. If re-using tip, keep the plunger fully depressed and lightly touch off on a clean, lint-free wipe to assure all drops are removed. If an occluding bubble develops, replace the tip with a new, clean tip.

4. **Avoid excessive pre-wetting:**

When applying a new tip, it is necessary to first pre-wet the tip once before beginning work. Some procedures may suggest multiple pre-wetting or 'humidification' cycles, but this is not effective at volumes below 2 µL and can increase chances of an occluding bubble forming.

5. **Use appropriate equipment and take environmental conditions into account when verifying performance or calibrating:**

When working at volumes below 10 µL, a scale sensitive enough to measure mass to 6 decimal places must be used to accurately measure the small amounts of liquid. The weighing vessel should be covered and protected from convective currents to ensure evaporation rate is constant. Effects of varying ambient conditions (temperature, humidity and pressure) should be factored into measurements using the proper conversion factor (Z-factor). See the Performance Verification document in the Support section of our website for more details.