Are you really pipetting 1 µL? How deep you dip the tip during aspiration and how you dispense can easily throw you off 10 - 20%. This study uses 2 powerful technologies to delineate these 2 collusive errors and show how you can pipette tiny volumes like 1 µL accurately.

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April 23, 2015 A Differential Pipettor was calibrated to 0.90 uL (by both gravimetrics and the Artel PCS) and used with Little Squirt tips. Sample was aspirated holding the pipettor substantially vertically and immersing the tip shallowly -- 1-2 mm below the surface of the sample --and then deeply -- 6.3 mm (1/4 inch). Dispensing was done into the PCS vial contact-free ("Blastoff"), delivering only the volume that was aspirated inside the tip. Then the sample was dispensed by conventional touchoff-and-drag.

Run #	1	2	3	4
Aspiration tip immersion	shallow 1-2 mm	shallow 1-2 mm	DEEP 6.3 mm	DEEP 6.3 mm
Delivery technique	Blastoff	Touchoff	Blastoff	Touchoff
Mean uL	0.888	0.911	1.063	1.087

The data from the 4 combinations of aspiration depth and means of dispensing is shown with graphics and color coding.

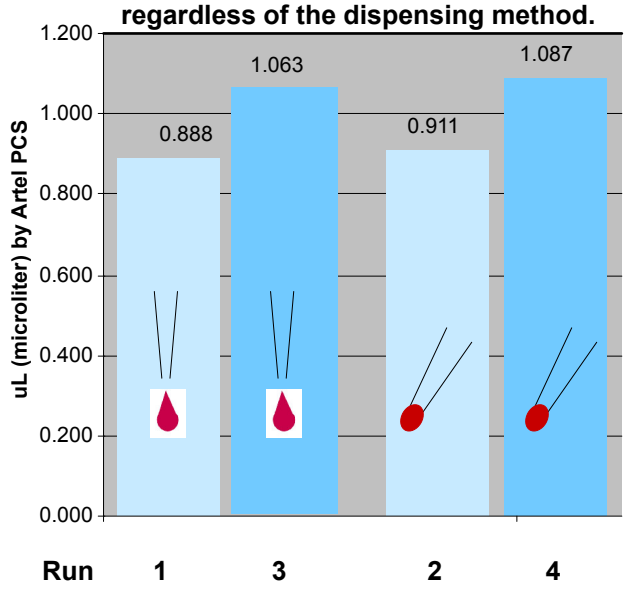
Contact-free (Blastoff)

Touchoff-and-drag

shallow dip

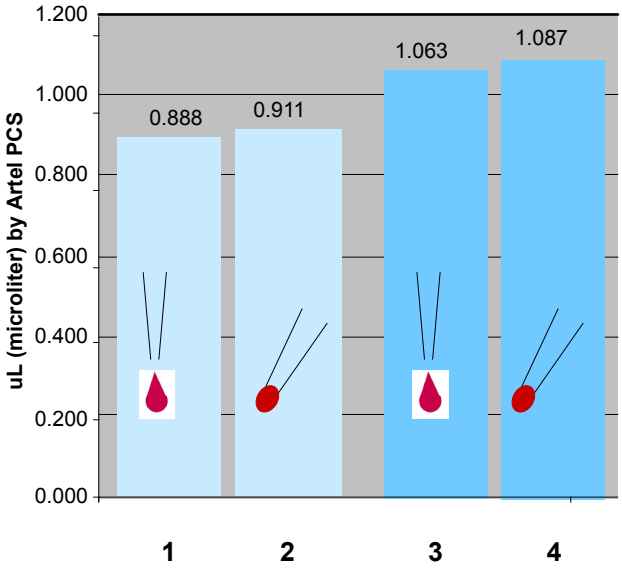
deep dip

GRAPH 1 Dipping unduly deep to aspirate increases aspirated volume by gravity regardless of the dispensing method.



Deep Dip increase with	uL	%
Contact-free (Blastoff) dispensing	0.175	19.7%
Touchoff-and-Drag dispensing	0.176	19.3%

Graph 2 Dispensing by Touchoff & Drag wicks-off additional volume from the outside



Touchoff increase with	uL	%
Shallow aspiration dipping	0.023	2.6%
Deep aspiation dipping	0.024	2.6%

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For those who are beginning to follow our SCIENCE section, the prior (April 17, 19 and 20) study (aka Science 1) used our clean contact-free dispensing to confirm the well-know fact that Mother Nature's powerful gravity and other hydrostatic effects cause more volume to be aspirated when one dips the tip deeper than needed for aspirating small samples. This study of April 23, 2015 uses an aspiration dip that is deeper and more consistent to fortify the prior baseline and to ADD the 2nd key variable of contact-free vs touchoff dispensing.

The data shows the major volume increase error from dipping the tip unduly deep during aspiration (the 900 pound gorilla). It also shows the additional significant volume from external "wickoff" of sample outside the tip (hefty chimp if you crave image symmetry) from conventional Touchoff-and-drag delivery -- the extra unintended sample clinging to the outside of the tip that is left behind by clean contact-free Blastoff dispense. Though not surprising to anyone versed in pipetting, this is probably the first time that actual quantitative data on these two very common sources of error has been published, let alone in combination. We had the advantage of being able to harness two powerful technologies to get at this. One is the Differential Pipettor, uniquely able to dispense these small volumes cleanly contact-freed but which can also deliver in the traditional touchoff-and-drag way, to compare the two directly. The other is the Artel PCS dual-dye ratiometric measuring system that gets around many evaporation problems when measure such small volumes. We used the same Differential Pipettor and our same Little Squirt tips to aspirate after correct shallow and deep dipping -- and to dispense both by our contact-free method and the traditional touchoff-and-drag method to see any difference. The value shown for each of the 4 run types is the average of 19, 32, 11 and 7 points each respectively.

The Differential Pipetting contact-free ("Blastoff") dispensing values accurately show what was aspirated inside the tip, and the traditional Touchoff-and-Drag dispensing gives a larger result because of the liquid that clings to the outside of the tip during aspiration that is “wickoff" during delivery. When the tip is dipped deeper during aspiration, and both methods therefore start with more aspirated sample inside the tip, the pattern is similar. This information should assist practical perspective on minimizing errors and optimizing accuracy when pipetting at the low or sub-microliter level.

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