Draft. Not Final.

Nanoliters, Where and Why.

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Ten years ago we patented a simple technology that we call induction based fluidics (IBF) that allows one to dispense or dispense and treat liquids non-touch in the uL, nL and pL regime. In IBF, one inductively charges liquids in a manner similar to that done by Millikan in his famous experiment to measure charge of the electron. IBF causes discrete liquid drops (not sprays) to fly to targets that can be TLC or MALDI plates, human beings or parts thereof, plants or animals or instruments like mass or optical spectrometers. Unlike, electrospray, a conductive, dispersive technique, IBF does not have faradaic processes and it is not dispersive. We can shoot liquids in a straight line, and put them where we want them without dispersion (dilution). That is a big deal.

Of course, the nanoliter regime offers many practically important advantages to users in labs everywhere. Firstly, the ability to manipulate nL quantities of liquids affords a user lab the ability to save 10 to 1000x on the cost of solvents, obviously. Moreover, by handling nLs one can make experiments with toxic chemicals, radiological or biological agents or other liquids much safer. Also, by using nL as opposed to uL and mL quantities of liquids, one can dramatically reduce the cost disposing liquids in regulated manner. Furthermore, using nLs allows one to use smaller hoods saving huge amounts of energy wasted in large hoods. So, we assert that using nLs in experiments is an exceedingly green practice and a win for the user and the planet, prima facie. Moreover, IBF allows one to do simple and exciting "new things" as described below with liquids in a simple manner that can be taught to technicians and others rapidly. One can easily learn to dispense nLs in a matter of minutes for many applications.

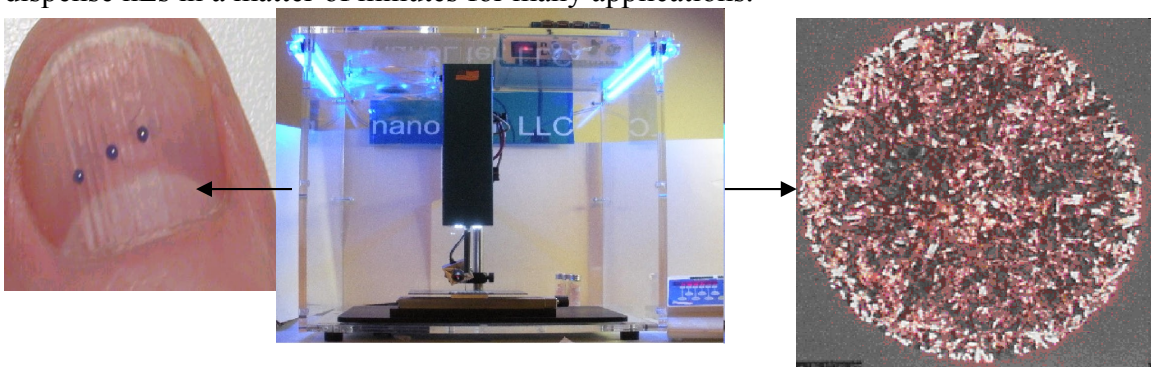

Figure 1 nanoLiter Cool Wave© dispenser "flys" directed nLs to humans or creates homogenous 50 nL dispensing of polymethylmethacrylate, courtesy of Professor Julie Harmon USF.

This attribute arises because IBF can be appended to common devices like microliter syringes, pipettes, and pumps of many types, simple devices, that are used to today, in

every lab in the world. For example, IBF can be used to generate homogenous MALDI depositions that afford a literal 10 to 100x increase in MADLI sensitivity as published in the peer review literature by T. Tu , M. L. Gross and J. Harmon for proteins, peptides and synthetics polymers (1,2,3). Or one can use our nanoLiter Cool Wave© dispenser to fly liquids to humans, test animals, teeth and even fly viscous liquids up! Alternatively, IBF can be appended to chips or instruments generally considered higher tech and as such IBF has a massive application space in low and high technology applications.

In fact, this year a number of worlds first have been demonstrated using IBF. For example, we reported at LabAutomation 2010, a 384 channel, uL and nL parallel dispensing onto and into MALDI and microtiter tape targets in 1 millisecond, using one source of energy. We believe that this is the fastest technique in the world (4), as we note that the same core IBF device could dispense 1536 or much higher number of channels. Given this attribute, IBF is likely the lowest cost per channel parallel dispensing, SPE, LC technology, as well.

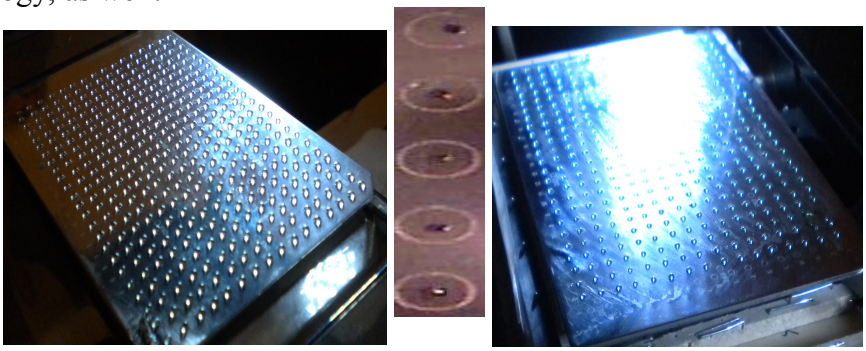

Figure 2. 384 channel parallel 1000 nL and 200 nL dispensed in 1.0 millisecond using an IBF modified Roche 384 Poly-pipettor, patent pending uL/nL tips and nanoLiter Cool Wave© electronics and single channel ca. 4, 30 nL liquid depositions (middle) from our nanoLiter Cool Wave© dispenser in Figure 1. Visit nanoliter.com.

At ASMS this year along with Drs. C. Cody and J. Dane of JEOL, we demonstrated that by using nanoliters in our IBF nL pipette tip and a JEOL AccuTOF-DART MS, we showed a 10 to 100 x improvement in detection limits for prescription and drugs of abuse (5).

Also this year, as published by J. Jarecki et al at the University of Wisconsin in ACS Chemical Neuroscience the ability to dispense nanoliters onto single cells and to identify six novel and other proteins was demonstrated (6). This occurred as T. Brewer, C. Szakal, and G. Gillen of NIST published an article where our nL device was used to dispense nLs of the viscous liquid glycerol on top of other chemicals and they showed a 100 to 1000 increase in SIMS sensitivity as published in RCM for cocaine and RDX (7)! And in 2008/9 in the first application of IBF at NIH, it was estimated that in negative ion, linear mode that a 100x increase insensitivity allowed for a never before identified post translationally modified tubulin in actual brain cancer samples!

In fact, in experiments conducted a decade ago we realized and subsequently demonstrated under and R&D agreement for Dr. Bill Davidson* former Director of R&D

of MDS Sciex that IBF could do parallel LC. We also knew that we could increase MALDI sensitivity as very simply, we could make concentrated, homogenous depositions. That is we could make deposits that were nothing but "sweet spots." This was in contrasted to the poor practice often called the dried (or dumb) droplet method. That stated we have been pleasantly surprised at the number of applications showing what we predicted for MALDI being observed for SIMS and DART MS techniques from a simple device, one that could be used for nanoliter pipetting or to help glues things together (like tissue?).

We will report this fall at ASMS's Asilomar meeting, and next year at MSACL, LabAutomation, Pittcon and ASMS why we believe that nL depositions are showing great increases in sensitivity of multiple MS techniques. One major reason that we get what Dr. Chip Cody called "more from less" is due to a number of factors. Perhaps, the most important issue being that we get less ionization suppression by using nLs as opposed to uLs. This is probably because as Drs. X. Fan and K. Murray have shown in their ASMS 2009 poster that showed femtosecond analysis of MALDI laser plumes, one can create many particles and other "stuff" in a mushroom cloud when blasting deposits with lasers (8). These entities are very large, as are drops, ion clusters produced in these experiments and probably destroy the analytical ion of interest in a number of ways. We speculate then that nL depositions yield more product ion as compared to uL deposition as there's less analytical ion destruction. That is, in using nLs, we have more analytical ions that are as Karas put it "lucky survivors." Of course, it is more complicated than that, but we are amazed at how many ions we apparently produce across techniques using nLs. It is a very counter intuitive result, but very real.

In conclusion, we see IBF as an intelligent way to handle liquids. IBF could eventually exist in most labs in the world just as microliter syringes, pipettes and pumps do today. IBF is a simple, green technology that can allow every lab in the world the ability to manipulate in a very cost effective manner, 3 more orders of magnitude of liquids easily. IBF empowers users to do exciting "new things" that are also green as it saves precious samples (evidence) and money on expensive chemicals and laboratory energy.

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* We were saddened to learn that Dr. Bill Davidson passed recently. Bill was in our estimation for many years the single best scientist, businessman, in the world.

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