Nanoliter

MS Sample Handling: Sensitive ESI & MALDI via One **ELECTROSTATIC** Approach, IBF.

(Electrospray MS “**With” And** “***Without”*** a Cone-Jet. Patented, Pending Technology)

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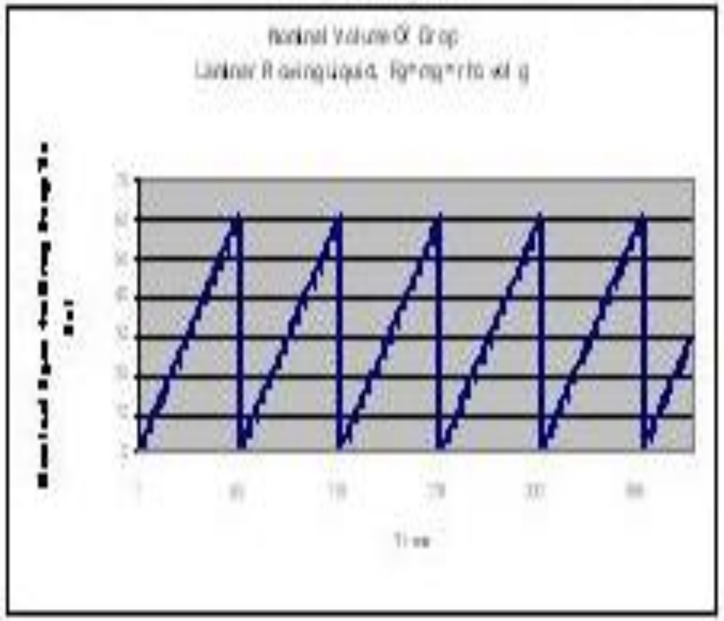
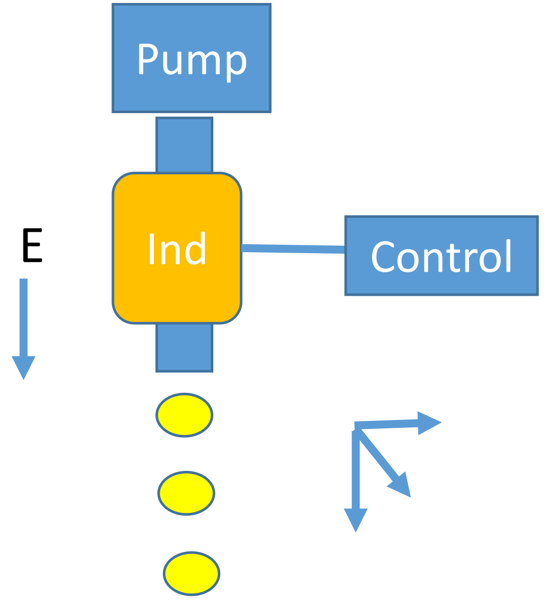
One Flowing Inductive Arrangement Cartoon.

One Common Circuit

$$V = ((\pi r^4 P)/8nl)t$$

Droplet Volume Grows & Collapses When Energized.



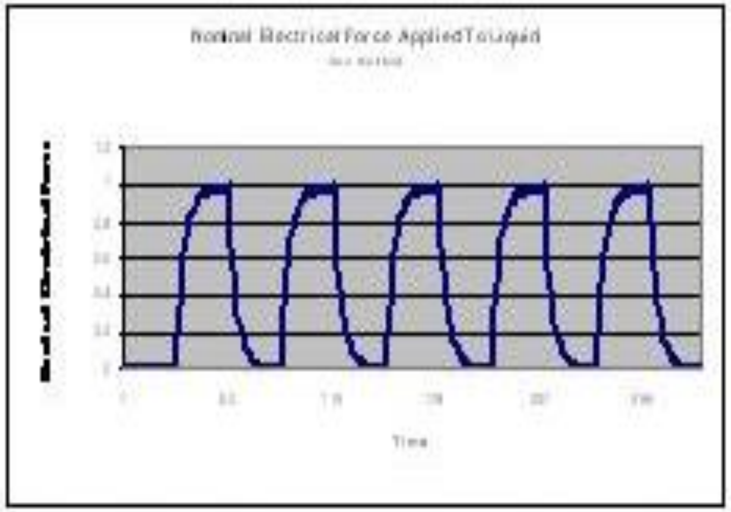


Droplet

$$F = qE$$

$$q = q_0 e^{(-t/\lambda)}$$

Droplet Shot Off Capillary Tip



Typically….
Gaussian surface
kV applied,
Low nL/sec flow
Scan time, 1 s or faster
2 mass decades

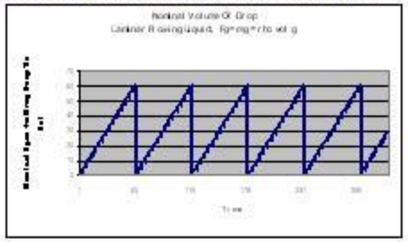
Not presenting a-m, amplitude modulation details.

https://www.youtube.com/watch?v=MefPfwrY7cw

IBF Theory

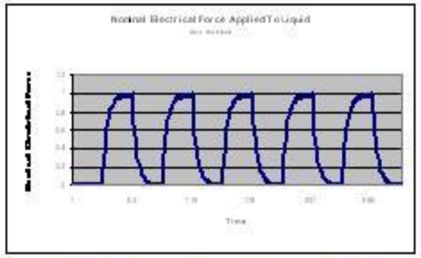
One IBF device, a flowing or stop flow system. There are many IBF embodiments

The liquid volume passing through a tube is given by the Hagen Poiseuille equation. The volume of fluid (*V*) that flows down a small-diameter capillary tube per unit of time (*t*) is proportional to the radius of the rube (*r*), the pressure pushing the fluid down the tube (*P*), the length of the tube (*l*), and the viscosity of the fluid n. Note v is linear in t.



$$V = ((\pi r^4 P)/8nl)t$$

Since electric fields can be rapidly toggled on and off, with great accuracy and precision, and since F= qE, the forces on liquid drops can be changed rapidly. Because F is a vector, we can direct the drop as well.



$$F = qE$$

For a charged drop with initial value, q0, with th e relaxation time, λ, where λ = (ε0εr / κ) where ε0 is the dielectric constant of free space, εr is the relative permittivity and κ is the solution conductivity and t is time, we have q defined.

$$q = q_0 e^{(-t/\lambda)}$$

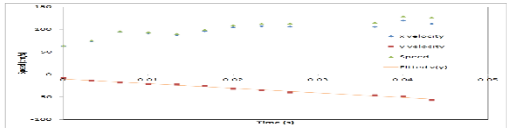
Now, a charged liquid drop in an electric field, cannot only experience the qE force, but of course it experiences, different forces as well in the atmosphere in x, y and z space depending on the specifics of the system. Using standard, well known physics, Newton's 2nd Law, we can equate the forces (electric, drag, buoyant, gravity and coulombic) acting on a drop to those acting in the direction x as,

$$F_x = m(a_x) = m(dv_x/dt) = F_{elec\,x} + F_{drag\,x} + F_{buoyant\,x} + F_{grav\,x} + F_{coul\,x}$$

The same can be written for the y and z coordinates. Then with accurate model equations for Fy and Fz, we can actually **calculate the trajectories** of the drops (distances of travel, d) of the drops at any time t, knowing the initial position of the drop and that

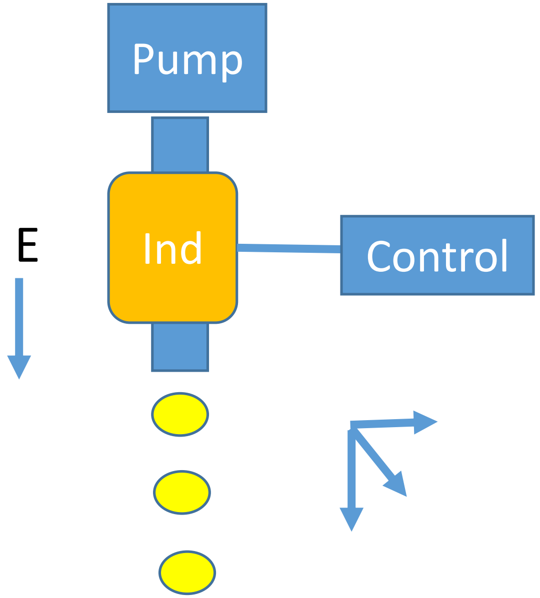
$$V_x = dx/dt,\ V_y = dy/dt,\ V_z = dz/dt$$

$$V^2 = V_x^2 + V_y^2 + V_z^2.$$



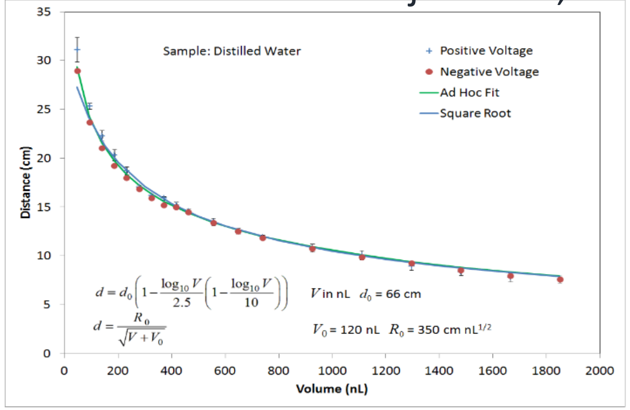
https://www.youtube.com/watch?v=MefPfwrY7cw

One Common Circuit

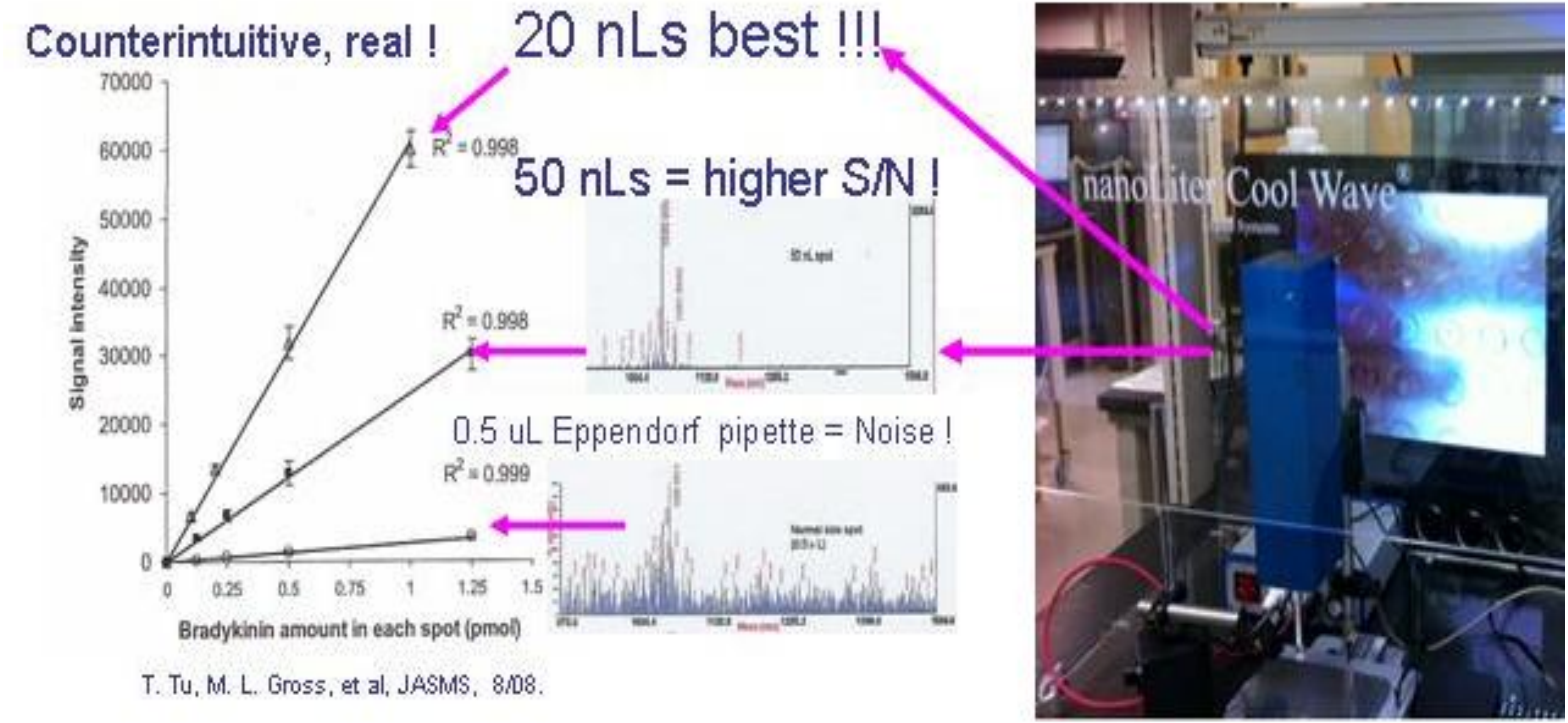


Droplet

+/- Droplets follow the same trajectories,17.

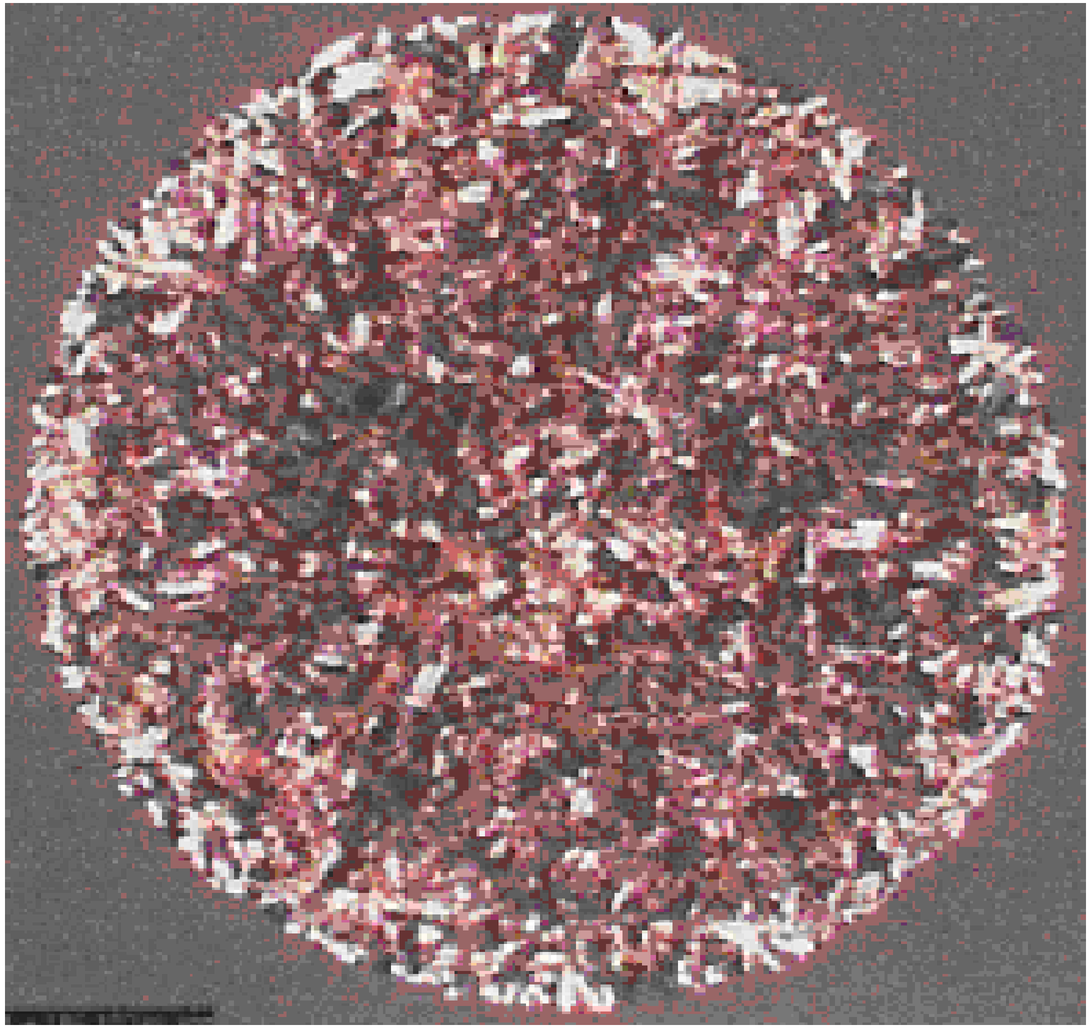


nanoLiter Dispense of Bradykinin on to MALDI Plate **T. Tu & Prof. M. Gross, et al , WUSTL**



Excellent PMMA Crystals. nLs Typically Yields 20 x or More Sensitivity Increase.

Prof. J. Harmon, et al, USF



100 nLs PMMA Deposition

C:\users\asauter\download\nanoliter25.wmv

nL dispensing yields excellent crystals
& > 20 x Increase in sensitivity or more
For MALDI and SIMS typically.!

< Cross Polarized hV Image.

Note poor 0.5 uL “crystals,” and high MS noise.
Note spatial concentration of sample EtOH/H2O.

Dr. Gillen, NIST Showed nLs of Viscous Liquid Improves SIMS Sensitivity by 100x, RDX & Cocaine.

Dispensing nLs of viscous glycerol droplets over inkjet, tetrabutyl ammonium iodide + analyte deposits, C60+, dynamic SIMS.

RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2010; **24**: 593–598
Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/rcm.4423

RCM

Method for improved secondary ion yields in cluster secondary ion mass spectrometry†

Tim M. Brewer*, Christopher Szakal and Greg Gillen
Surface and Microanalysis Science Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA

Received 23 October 2009; Revised 10 December 2009; Accepted 14 December 2009

A method to increase useful yields of organic molecules is investigated by cluster secondary ion mass spectrometry (SIMS). Glycerol drops were deposited onto various inkjet-printed arrays and the organic molecules in the film were rapidly incorporated into the drop. The resulting glycerol/analyte drops were then probed with fullerene primary ions under dynamic SIMS conditions. High primary ion beam currents were shown to aid in the mixing of the glycerol drop, thus replenishing the probed area and sustaining high secondary ion yields. Integrated secondary ion signals for tetrabutylammonium iodide and cocaine in the glycerol drops were enhanced by more than a factor of 100 compared with an analogous area on the surface, and a factor of 1000 over the lifetime of the glycerol drop. Once the analyte of interest is incorporated into the glycerol microdrop, the solution chemistry can be tailored for enhanced secondary ion yields, with examples shown for cyclotrimethylenetrinitramine (RDX) chloride adduct formation. In addition, depositing localized glycerol drops may enhance analyte secondary ion count rates to high enough levels to allow for site-specific chemical maps of molecules in complex matrices such as biological tissues. Published in 2010 by John Wiley & Sons, Ltd.

Recent advances in molecular imaging mass spectrometry have led to an increased interest in the spatially resolved analysis of organic compounds. Specifically, techniques utilizing imaging mass spectrometry are resulting in important bioanalytical applications,[1–4] with secondary ion mass spectrometry (SIMS) playing a vital role for imaging

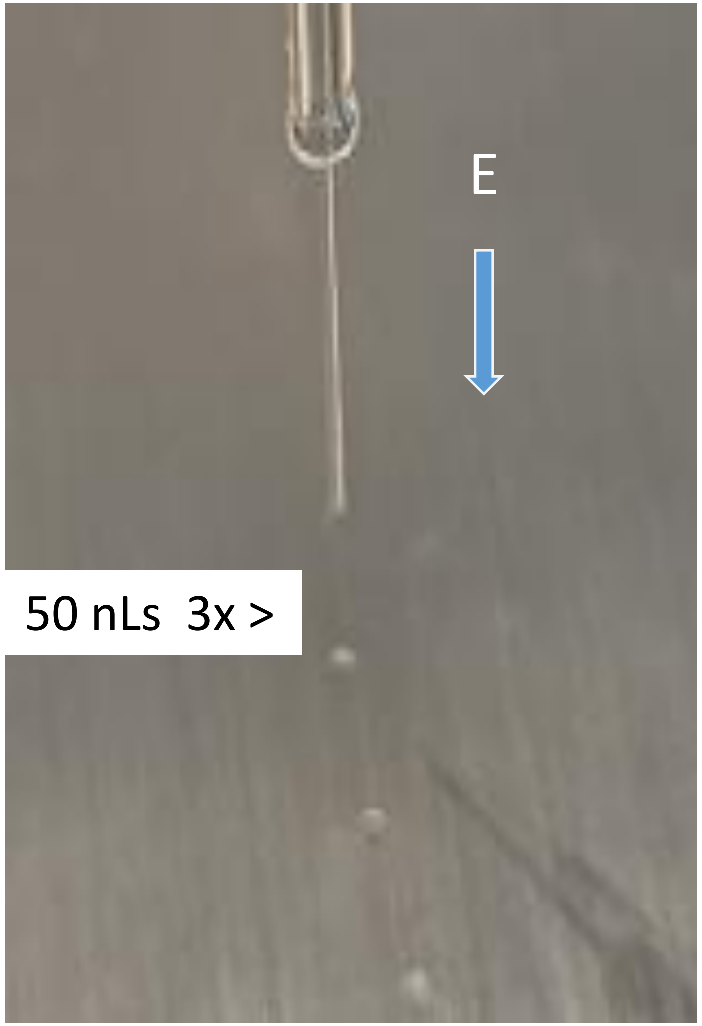
increase useful yields: sample modification and the use of cluster projectiles. An example of sample modification to enhance ion yields is through metal-assisted secondary ion mass spectrometry (ME-SIMS).[8,10,11] In this methodology, secondary ion yields are enhanced by deposition of a small quantity of metal on the sample surface.[12] The second

Single Channel Dispensing for DOD.

Used in 4 Classified DOD nL Dispensing Projects. Natick, ECBC, APG,MD, Dugway PG and Tyndall AFB.

nLs ONTO

ASMS Army Poster, APG MD.
http://www.nanoliter.com/armyposter.pdf

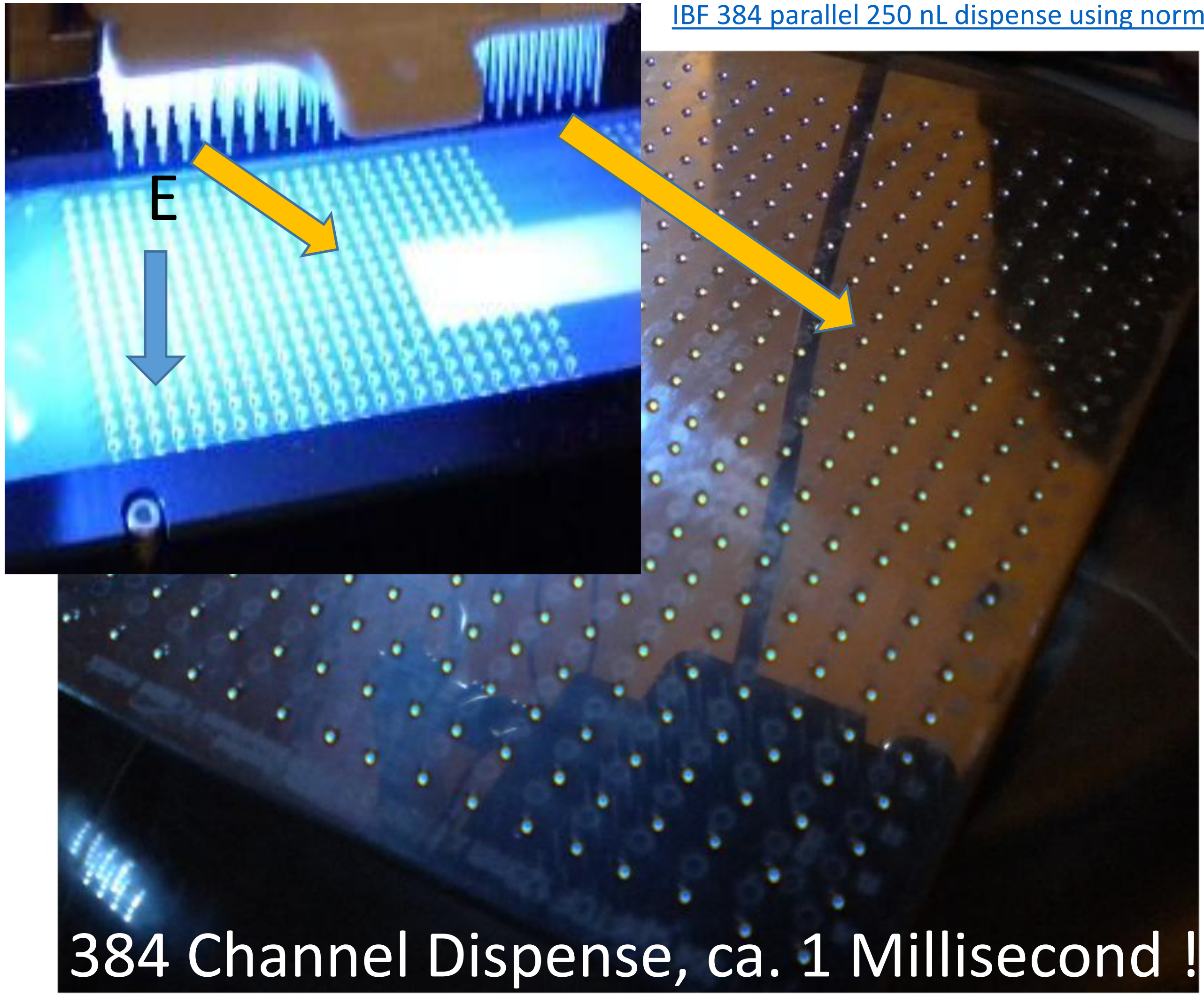


nLs INTO

3 EXAMPLES OF PARALLEL ROBOTIC MILLISECOND nL DISPENSING via IBF.

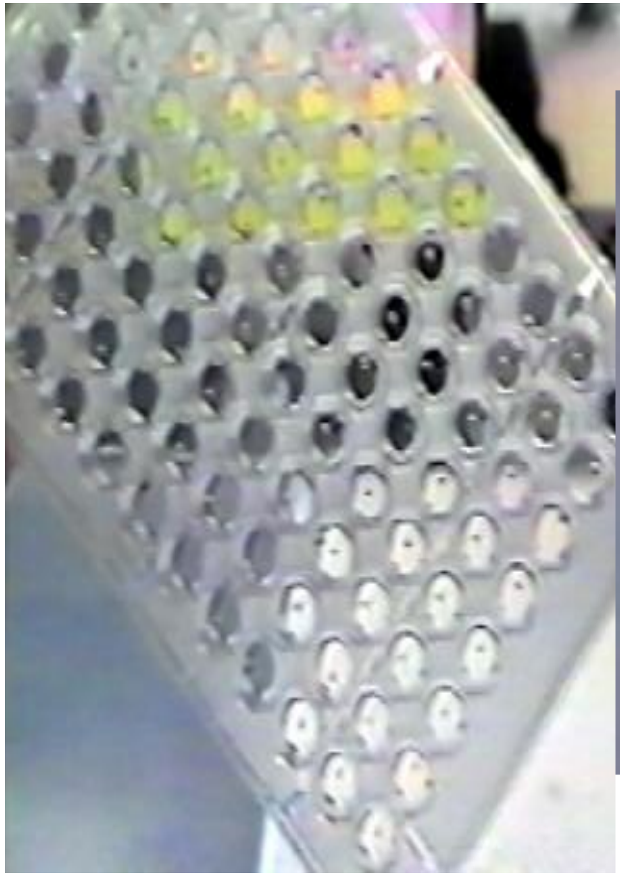
Highly Parallel 384 Channel 150 nL ***Directed*** Dispense (EtOH/H2O) Regular Tips. Drew Sauter

IBF 384 parallel 250 nL dispense using normal tips.



https://www.youtube.com/watch?v=jwaK2ntxybg

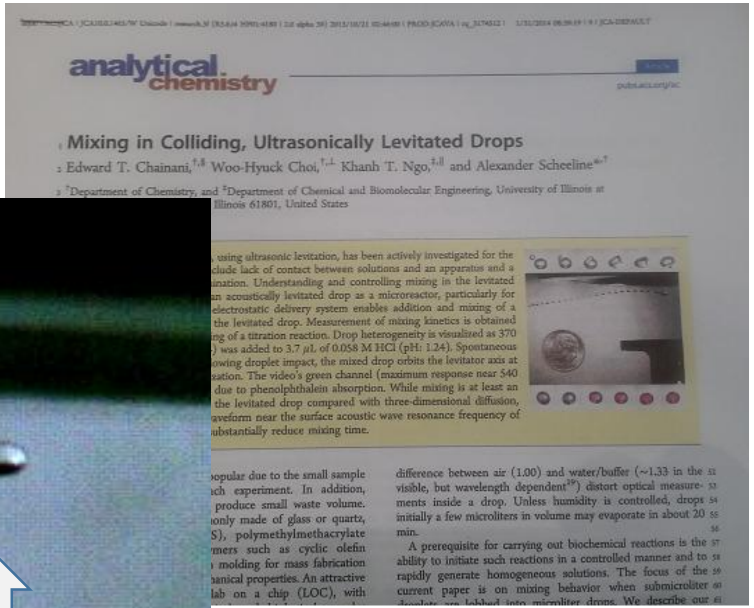
1 second/8 channel/50 nL EtOH/H2O



Single Channel Spark Alias
200 milliseconds/dispense.

Wall-less kinetics. Pure R&D.

Prof. Alexander Scheeline, et al, U. Illinois, Urbana.


analytical chemistry

Mixing in Colliding, Ultrasonically Levitated Drops

Edward T. Chainani,[†,§] Woo-Hyuck Choi,[†,⊥] Khanh T. Ngo,[‡,∥] and Alexander Scheeline[*,†]

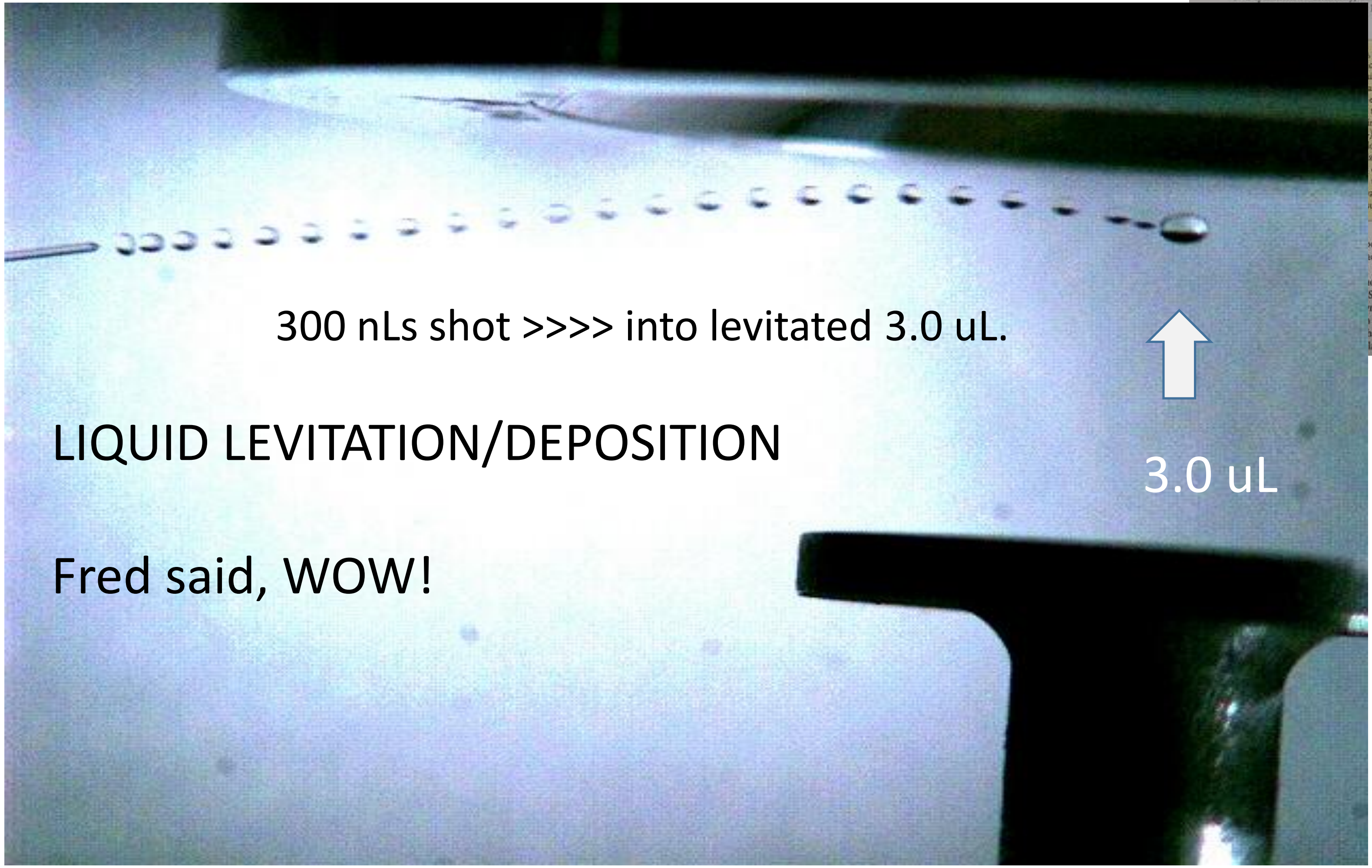
[†]Department of Chemistry, and [‡]Department of Chemical and Biomolecular Engineering, University of Illinois at ... Illinois 61801, United States

... using ultrasonic levitation, has been actively investigated for the ... clude lack of contact between solutions and an apparatus and a ... ination. Understanding and controlling mixing in the levitated ... an acoustically levitated drop as a microreactor, particularly for ... electrostatic delivery system enables addition and mixing of a ... the levitated drop. Measurement of mixing kinetics is obtained ... ing of a titration reaction. Drop heterogeneity is visualized as 370 ...) was added to 3.7 μL of 0.058 M HCl (pH: 1.24). Spontaneous ... owing droplet impact, the mixed drop orbits the levitator axis at ... zation. The video's green channel (maximum response near 540 ... due to phenolphthalein absorption. While mixing is at least an ... the levitated drop compared with three-dimensional diffusion, ... aveform near the surface acoustic wave resonance frequency of ... ubstantially reduce mixing time.

... popular due to the small sample ... ch experiment. In addition, ... produce small waste volume. ... only made of glass or quartz, ... S), polymethylmethacrylate ... mers such as cyclic olefin ... molding for mass fabrication ... anical properties. An attractive ... lab on a chip (LOC), with ...

difference between air (1.00) and water/buffer (~1.33 in the visible, but wavelength dependent[19]) distort optical measurements inside a drop. Unless humidity is controlled, drops initially a few microliters in volume may evaporate in about 20 min.

A prerequisite for carrying out biochemical reactions is the ability to initiate such reactions in a controlled manner and to rapidly generate homogeneous solutions. The focus of the current paper is on mixing behavior when submicroliter ...



300 nL shot into levitated 3.0 uL

. Edward T. Chainani , Woo-Hyuck Choi , Khanh T. Ngo , and Alexander Scheeline, Mixing in Colliding, Ultrasonically Levitated Drops, Anal. Chem., Publication Date (Web): January 24, 2014 Copyright © 2014 American Chemical Society, Manuscript DOI: 10.1021/ac403968d

More nanoLiter IBF Dispensing Applications & Results…..

(See video & reference links below.)

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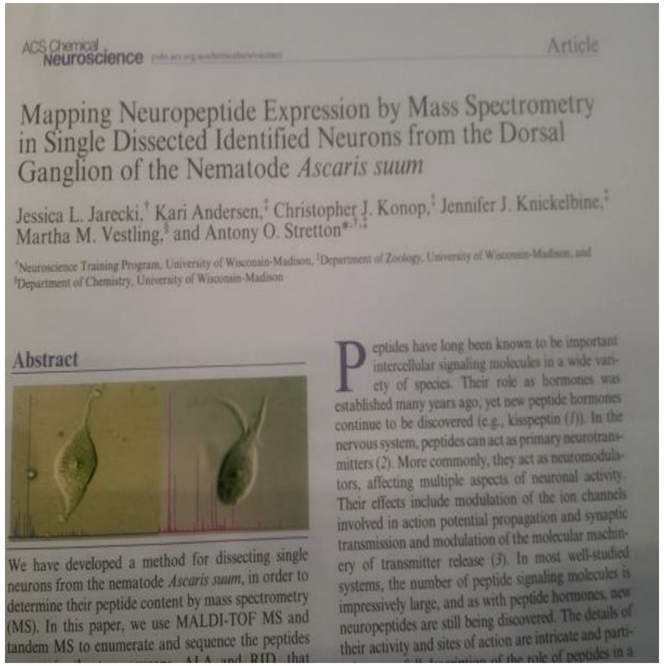
SINGLE CELL (Neuron) MALDI PAPER Wisconsin (Jarecki, Vestling, Stretton) identified 6 unique proteins via IBF MALDI.



ACS Chemical Neuroscience

Article

Mapping Neuropeptide Expression by Mass Spectrometry in Single Dissected Identified Neurons from the Dorsal Ganglion of the Nematode *Ascaris suum*

Jessica L. Jarecki,[†] Kari Andersen,[‡] Christopher J. Konop,[‡] Jennifer J. Knickelbine,[‡] Martha M. Vestling,[§] and Antony O. Stretton*,[†,‡]

[†]Neuroscience Training Program, University of Wisconsin-Madison, [‡]Department of Zoology, University of Wisconsin-Madison, and [§]Department of Chemistry, University of Wisconsin-Madison

Abstract

We have developed a method for dissecting single neurons from the nematode *Ascaris suum*, in order to determine their peptide content by mass spectrometry (MS). In this paper, we use MALDI-TOF MS and tandem MS to enumerate and sequence the peptides

Peptides have long been known to be important intercellular signaling molecules in a wide variety of species. Their role as hormones was established many years ago, yet new peptide hormones continue to be discovered (e.g., kisspeptin (*1*)). In the nervous system, peptides can act as primary neurotransmitters (*2*). More commonly, they act as neuromodulators, affecting multiple aspects of neuronal activity. Their effects include modulation of the ion channels involved in action potential propagation and synaptic transmission and modulation of the molecular machinery of transmitter release (*3*). In most well-studied systems, the number of peptide signaling molecules is impressively large, and as with peptide hormones, new neuropeptides are still being discovered. The details of their activity and sites of action are intricate and parti-

Jarecki, J.L.,Anderson,K., Konop,C.J., Knickelbine, J.J., Vestling, M. M. and Stretton, A.O.,Mapping Neuropeptide Experssion by Mass Spectrometry in Single Dissected Neurons from Dorsal Ganglion of Nematode Ascaris suum. ACS Chemical Neuroscience, 2010,1:505-519.

Millisecond Robotic Dispensing
for Spark Holland, Alias's Dispenser. Sauter.
https://m.youtube.com/watch?v=cv1xWiyDgoM

PTM of a Tublin (Glycosolation) found in brain cancer samples due to est. 100x increase in MALDI sensitivity.
NIH (**Yergey, A.**, (RIP)),
Personal com. 2008.

Parallel LC/MALDI of Dyes Demonstrated for **Dr. Bill Davidson,** Sciex. Offer to license, twice. Reorg killed the deal.

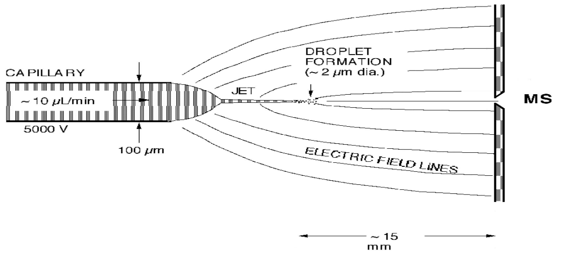
DART improved by 10 to 100x for analysis of drugs via IBF in 2010 at ASMS. **Cody, R**. et al, ASMS 2010 poster, Saple Preparation and Sample Presentation for Direct Analysis in Real Time (DART), Salt Lake City, UT, June2010. IBF Sample Prep, Presentation to DART, ASMS 2010 poster w/JEOL.

Genetech, Dr. Vatta, et al, Proteins and Peptide, MALDI 50 nL deposition ion current equals .1.0 uL deposition.
http://www.nanoliter.com/contactus.htm.

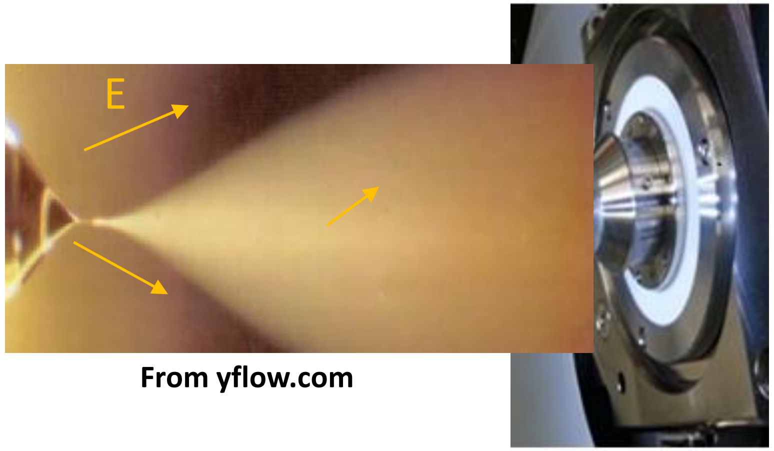
Traditional ESI Spray vs IBF “ESI” Droplets.

Traditional ESI.

Most sample is **<u>lost</u>** due to 1. E field dispersion and 2. like charge droplet repulsion.



Used With Permission of Professor Francis Beaudry

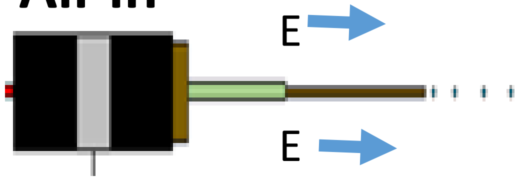


From yflow.com

Not to scale

IBF

No Large Cone-jet
All-in



Large cone-jet does NOT form!
So dispersive lines of force do not form !

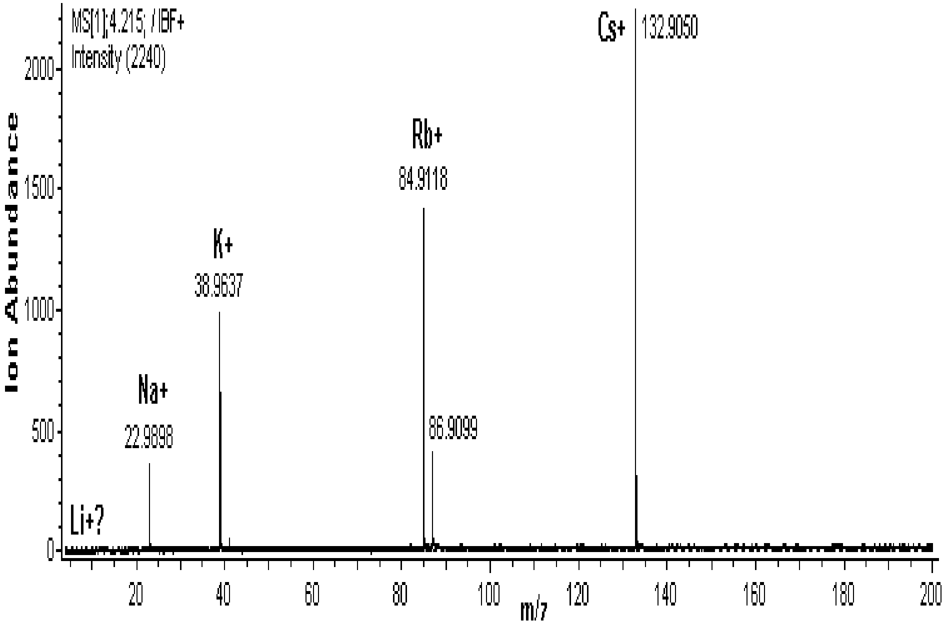
When I showed this to Professor Fenn, he said, ”Thank you for giving me something to think about.”

https://www.youtube.com/watch?v=skvG1oxdE9o

IBF Droplets & ESI Sprays Produce "Similar" MS For Alkali Metals, Oligonucleotides, (20 mers).

Equi-u-molar Alkali metals, LV EPA

IBF Droplet



Enthalpy of hydration of alkali metal cations and relative ion abundances. Acquired by our work and that published by Van Dorsslaer

Cation	Enthalpy of Hydration (kJ/mole)	% Relative Ion Abundance ESI, H_2O* 50% MeOH/H_2O	IBF,
Cs+	260	100	100
Rb+	290	SPRAY 60	63 Droplet
K+	335	20	44
Na+	405	7	16
Li+	535	2	0

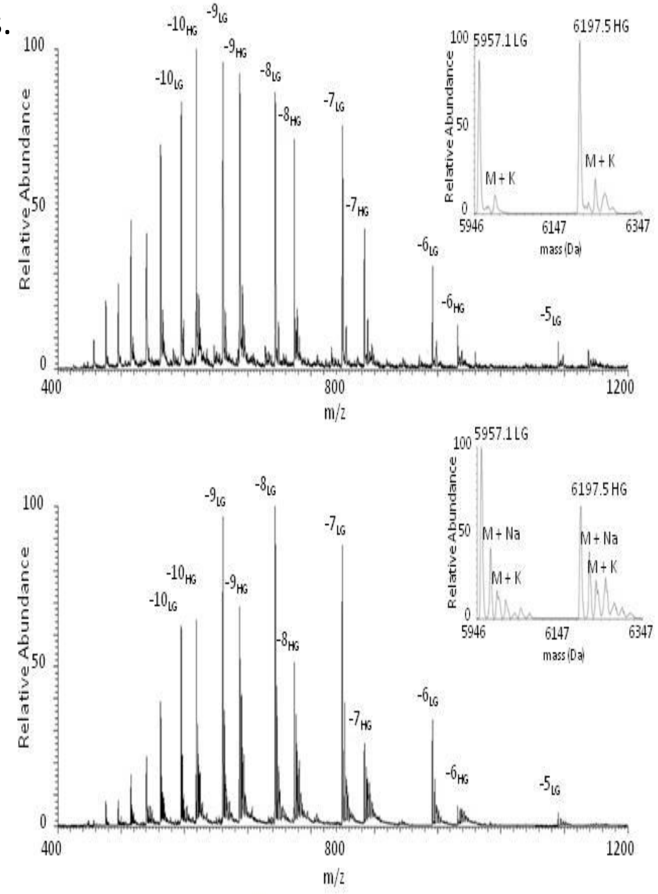
* E. Leize, A. Jaffrezic, A. Van Dorsselaer, JMS, Vol., 31, 537-544,1996. Q&D on the floor at Pittcon 2009.

Equimolar (5 pmol/uL) of oligonucleotides. UC

20-mer—LG (5'-ACA TCT CCC CTA CCG CTA TA-3') and 20-m-HG (5'-CGG CGG TGG CGG CTG TTGCT-3')… Promass Mass Deconvolution of molecular ions.

IBF Droplet

Spray



Mass spectra obtained from equimolar amounts (5 pmol/μl) of oligonucleotides 20-mer-LG (5'–ACA TCT CCC CTA CCG CTA TA–3') and 20-mer-HG (5'–CGG CGG TGG CGG CTG TTG CT–3'). Sample introduction in (a) droplet and (b) continuous mode at 5 μl/min. Inset shows deconvoluted molecular ions generated from ProMass software. Droplet mode operation minimizes the sampling bias related to nucleobase hydrophobicity. One explanation for this is that the nanoliter droplet produced by the IBF device undergoes fewer cycles of evaporation and fission than that produced in and ESI source.

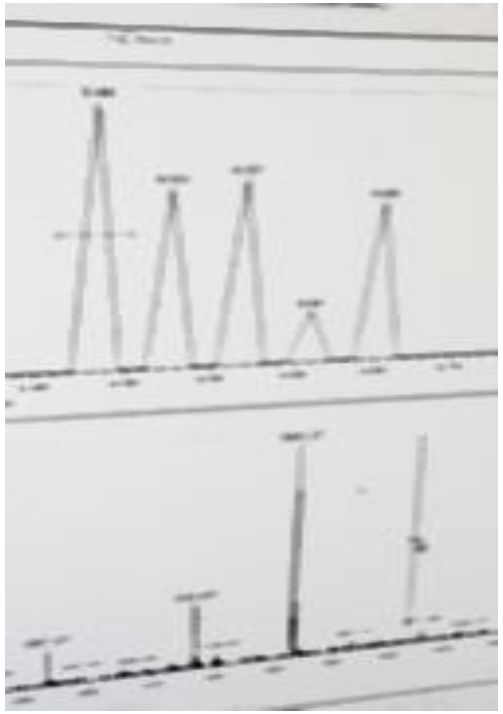
For Droplets, nL Peak Shapes, Very SHARP. Little Wall interaction, Very Different Systems. Helps Standardize Input.

Also, rate of programmed sample input can be 100-1000 x that of continuous spray. (Best Results = Energy Programmed.)

Precision can approach, ca. 5% RSD. ESI MS systems can handle much larger nL volumes quite easily. **Note nLs > fLs ,rapidly for desolvattion!**

Penicillin, +eV, TIC

Analogue Device

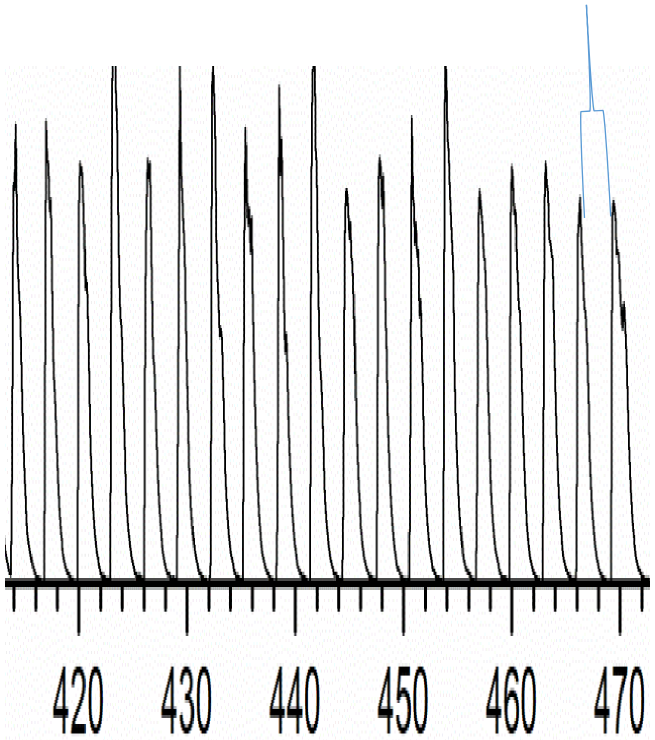


Manual infusion TIC. under-sampled, with poor ca. 3 s. injections. Unipolar, +eV. LTQ, TIC, NSC

Lanthanide tetra nitrate –eV ion

Digital Device

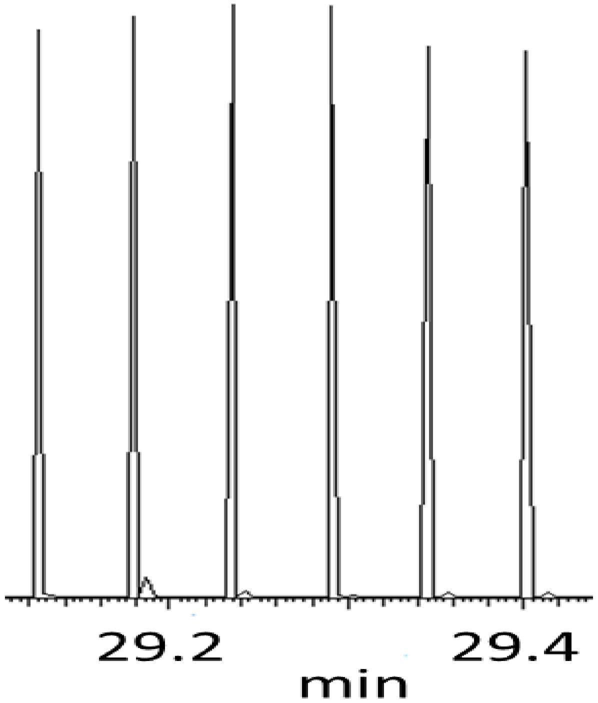
m/z 422.9, $[Lu(NO_3)_4]^-$ from 400 to 470, 3 sec/scan.



Digital bipolar 3 second shots. Bruker u-ToF. INL
http://www.youtube.com/watch?v=mzMDdFul6hE

MS/MS, 5 Nucleoside mix.

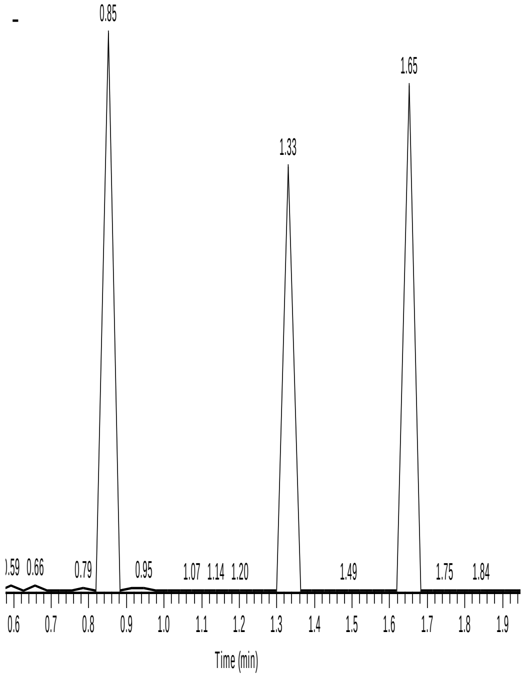
Digital Device



Infusion Bipolar Android Programmable Source, 2s.
+eV, LTQ, TIC, U Cin.

25 Pesticide Screen ca. 1 Hz

Digital Device



+ eV, TIC, LCQ MS via nL Prog Wave 75% fs, 50 nL, droplets. Ca. 1 % formic, Caltech, 8/18

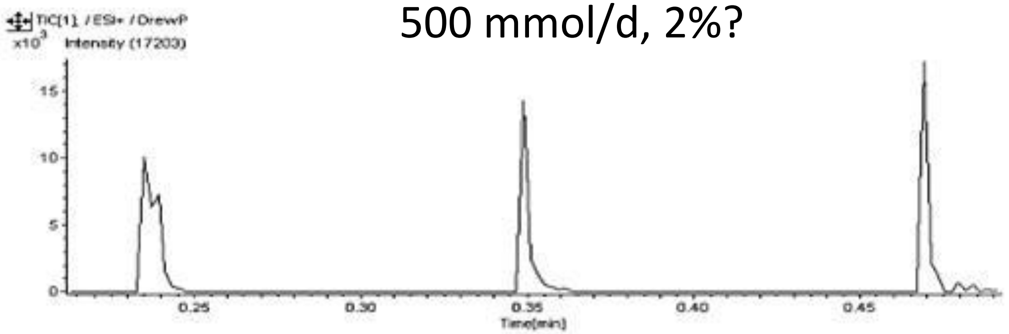
In all of the above examples a droplet, ca. 50/100 nL was shot at ca. 1 m/s yielding the sharp ion current peaks from the droplets.

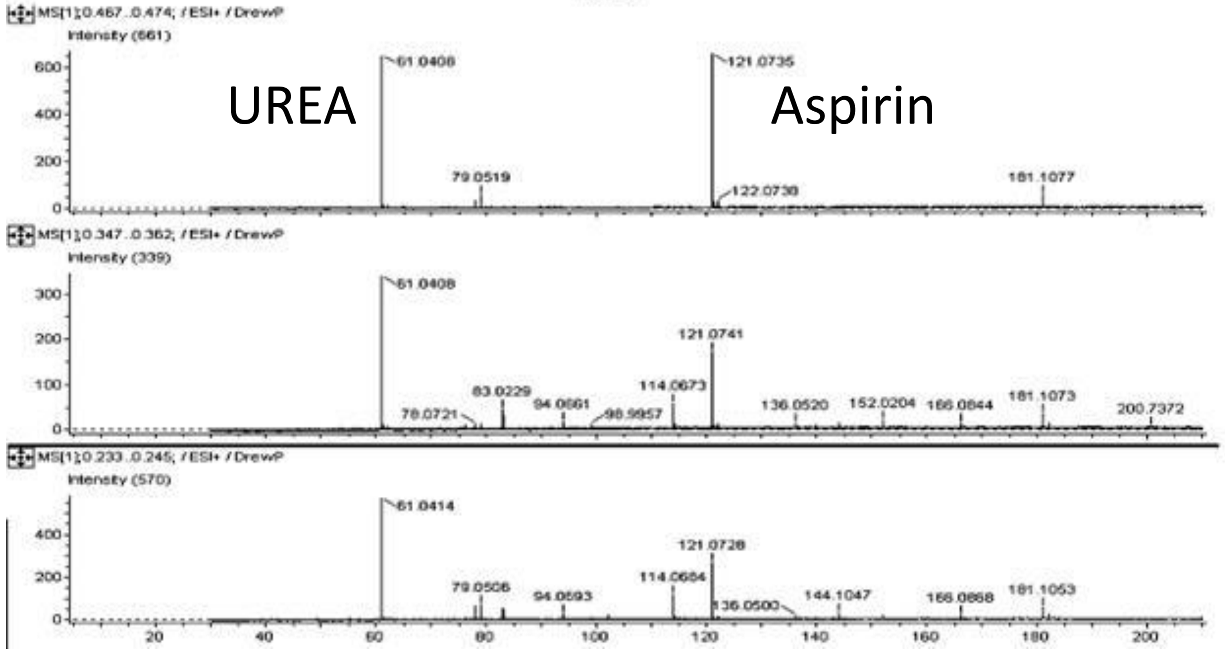
Very Early IBF data, + eV, reflectron, JEOL ToF, 10 Hz, 20-2000 amu.

IBF yields excellent dn/dt into source & hence reproducible di/dt!

Direct Urine Analysis TIC 3X.
Plus 3x, Urea + Aspirin, 50 nL mass spec.

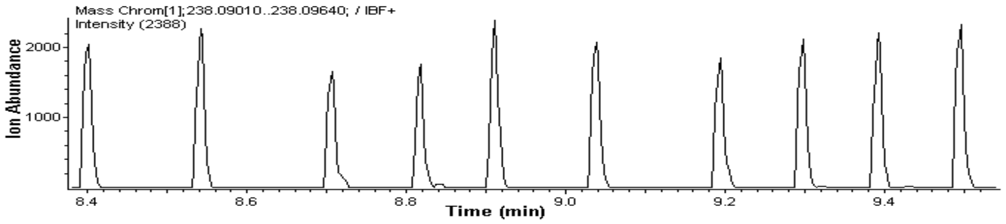
500 mmol/d, 2%?



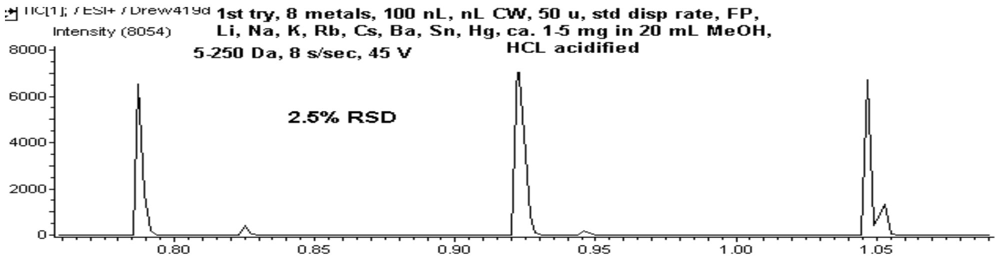


60 pg ketamine, 50 nLs,

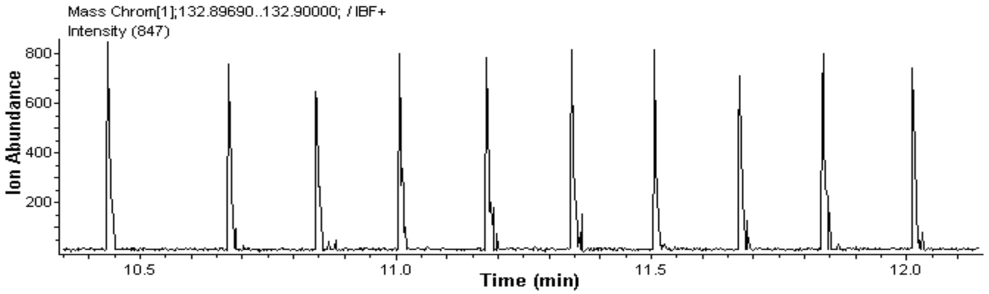
m/z 238, N=10 RSD = 11.0%



TIC, 50nLs, ppm metal mix, n=3, RSD 2.5%

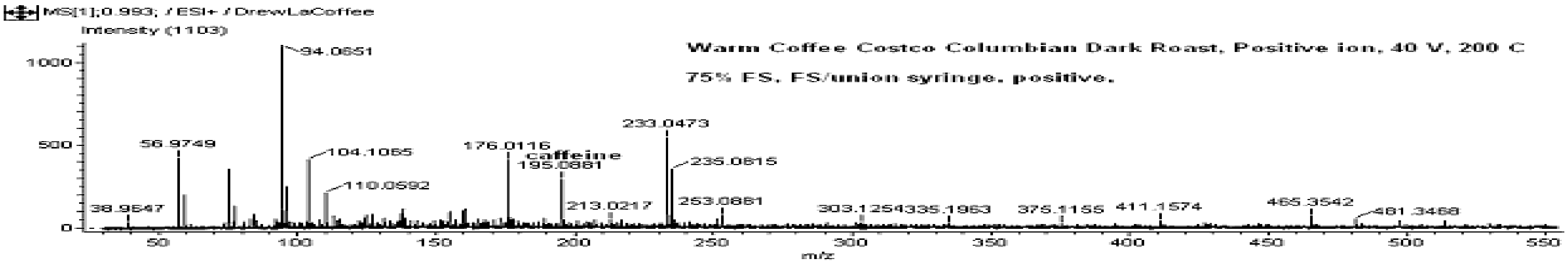


Cs+ n=10, 7.9%. m/z= 133, 50 nLs.,

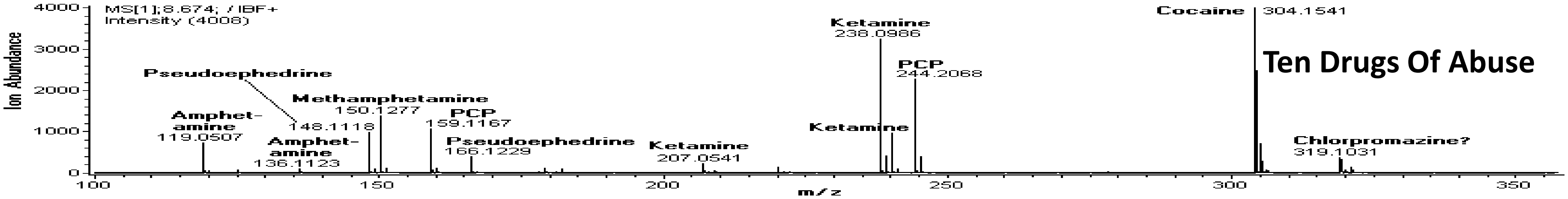


IBF Droplet Infusion, Examples of Mixtures Analyzed via JOEL TOF, +eV, w/ Stable Labelled Isotopes.

Coffee

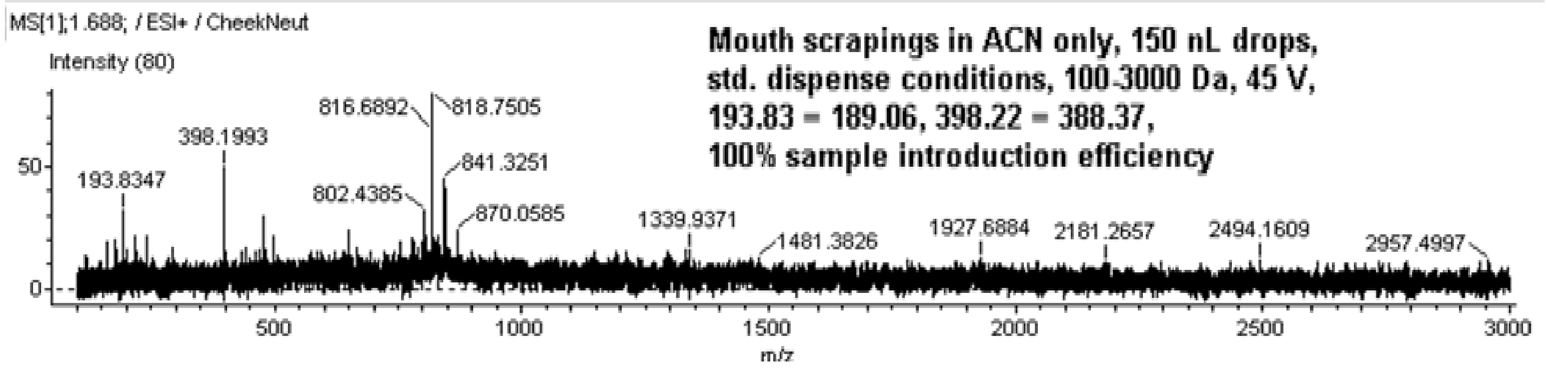


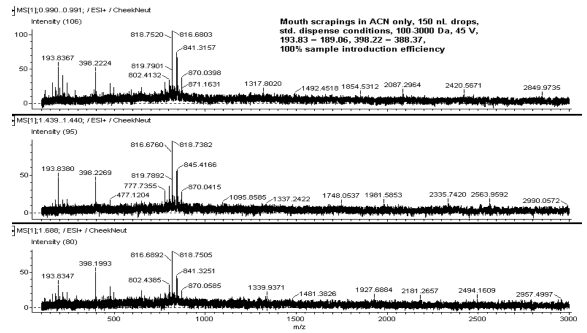
10 Drugs



Triplicate

Cells/Saliva



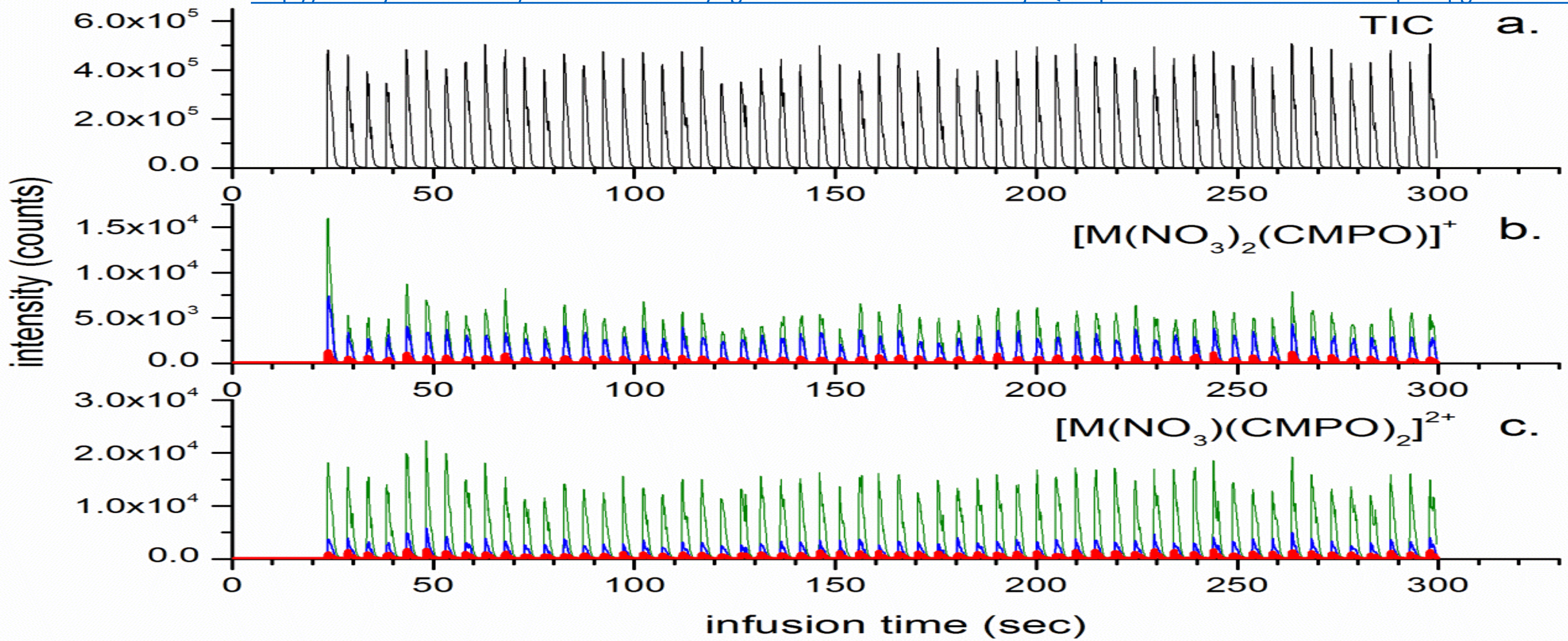


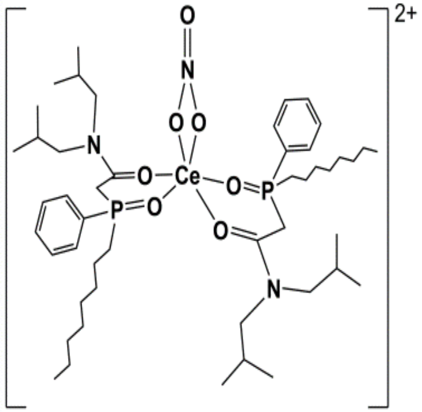
ENTIRE LANTHANIDE SERTIES OF ELEMENTS (CHELATES) ANALYZED FIA w/ PROGRAMMED IBF ENERGY, (Ce below.)

From Gaussian Surfaces, EtOH/H2O @ 17 nLs/sec for FIA, (1) CMPO, +eV, cNO3x –eV) .

Dr. G. Groenewold, et al, USDOE, Idaho National Lab

http://www.youtube.com/watch?v=cv1xWiyDgoM&context=C4caf0caADvjVQa1PpcFNGAEfH1ViRHiG8PuuZPdpYT4pgDS2M82k=





Positive ion profiles generated by individual drops. a. Total ion current. b. $[M(NO_3)_2(CMPO)]^+$. c. $[M(NO_3)(CMPO)_2]^{2+}$. Blue = Ce^{3+}, Green = Tb^{3+}, Red = Lu^{3+}.

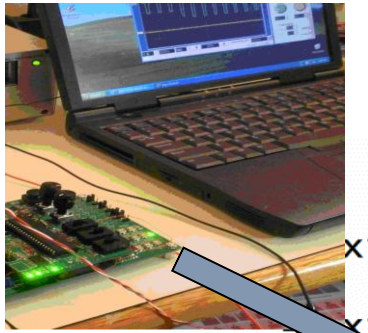
Groenewold, Gary; Sauter, Andrew; Sauter, Andrew, III, Rapid Analysis of Single Droplets of Lanthanide-Ligand Solutions by Electrospray Ionization Mass Spectrometry using an Induction Based Fluidics Source, Anal. Chem.published, ACS http://pubs.acs.org/doi/abs/10.1021/ac400863g?journalCode=ancham. E-mail: gary.groenewold@inl.gov. Poster presented ASMS 2013.

By programming the droplet energy, we can launch on command and we don't need to spray, placing 100% into the MS. Also, the background is very low aiding the attainment of fg and ag detection limits **WITHOUT** optimization and there's no faradaic processes, as the energy introduction process in inductive. For very rapid programmed energy, we can get streams, and if you want Note that this nanoLiter Cool Wave system has other proprietary issues that we cannot, now divulge`` CMPO = octyl.phenyl, (N,N-diisobutyl carbamoyl)tethy phosphine oxide).

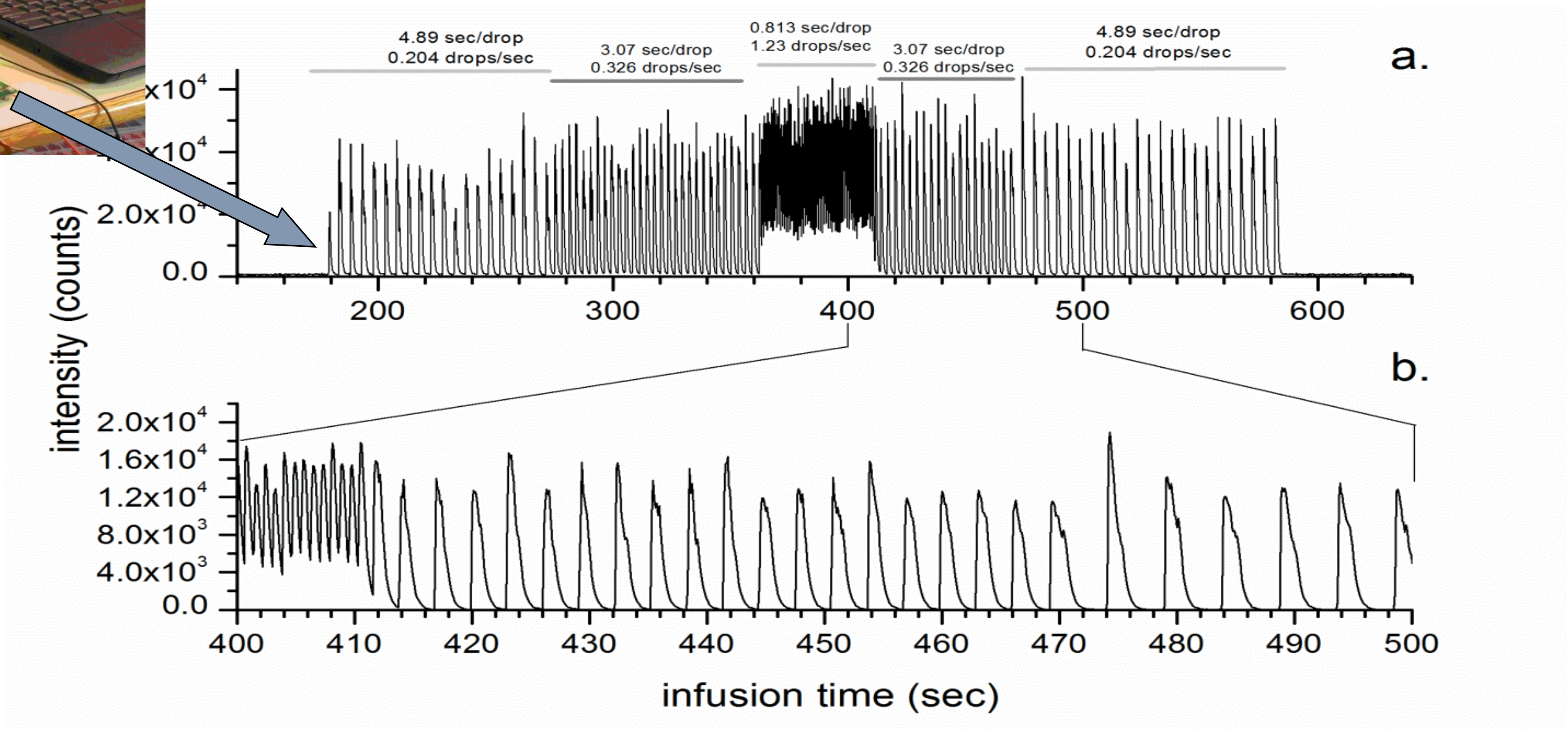
IBF Droplets, Fast (msec.), 100%, Input Efficient IBF Sample Introduction of m/z 422.9, $[Lu(NO_3)_4]^-$

See Analytical Chem. Papers (1,2).

100 determinations of 30 Solutions of +eV and – eV Lanthanide chelates analyzed in ca. 2 hours.

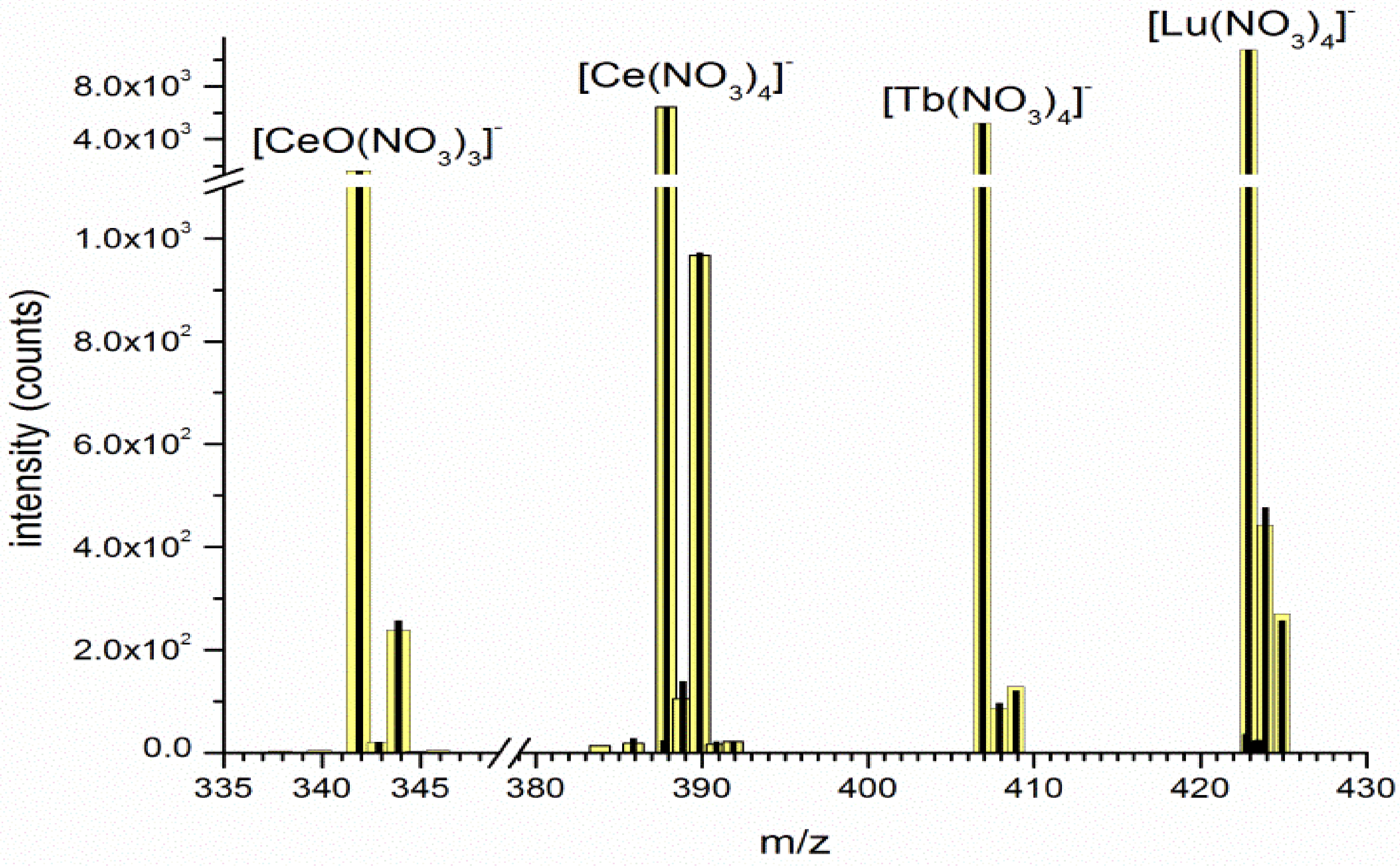


Ln-CMPO solutions, Digital control of pulsing, ~100 nL droplets, Anion mode. 17 nLs/sec.



Infusion profiles of a series of individual droplets. a. Total ion profile, showing five different frequencies. b. extracted ion profile of **m/z 422.9, $[Lu(NO_3)_4]^-$ from 400 to 500 sec.**

Accurate Isotope Ratios. Lanthanide Chelates. (Ce, Tb, and Lu) Infusion Only. No Plasma Needed !!! INL.



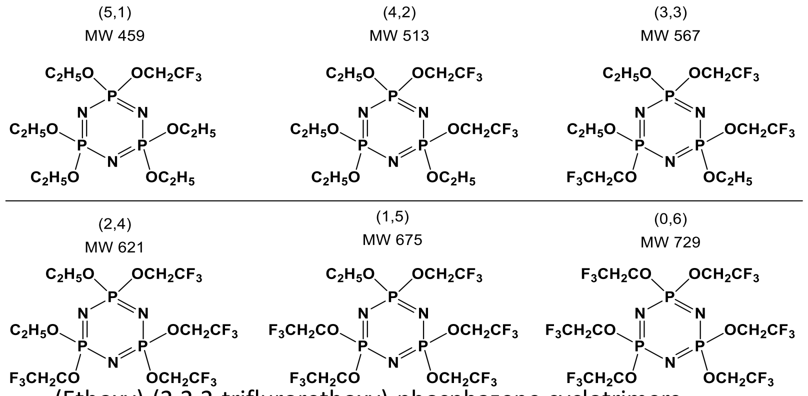
Comparison of abundances of minor isotopes with theoretical values. Black lines represent measured values. Yellow bars are the theoretical abundances of the minor isotopes. (16). IBF only, NO PLASMA!

Substituting the IBF source for the standard ESI source did not appear to compromise the function of the TOF mass spectrometer. The accurate *m/z* values for the nitrato clusters were within several ppm, and the relative abundances of the isotopic ions were very close to theoretical. In this work, the entire Lanthanide series of elements was determined in 30 solutions in +V and –eV at 100 determinations/ liquid in two hours. In one two day period 6400 determinations were made via IBF only sample introduction, via a Bruker u-ToF, WITHOUT A PLASMA (16).

nanoLiters of a Lithium battery electrolyte w/fire retardants shot directly into a HRMS @ INL.

Work of G. Groenewold, et al @ Idaho Nat'l Lab.

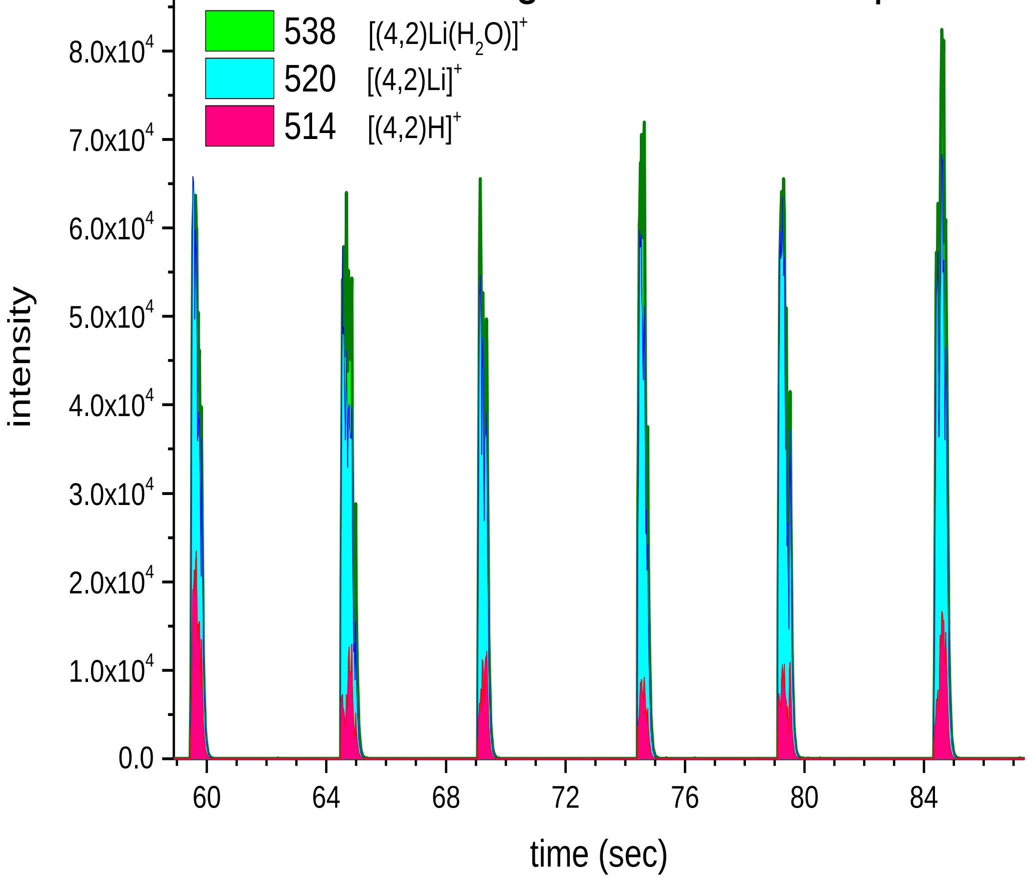
- Temporal profiles
  - 100 nL, manually launched, below.
  - 35 nL, field induced, @ 0.5 Hz, not shown

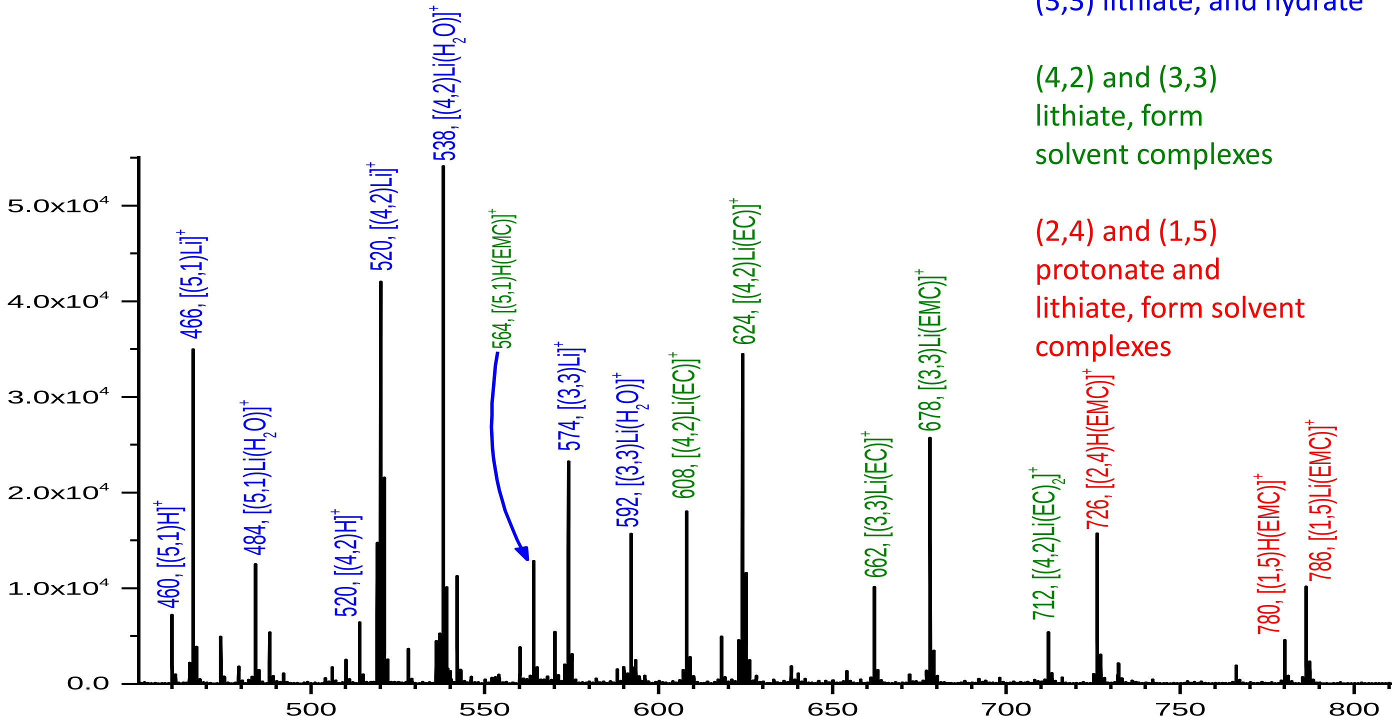


(Ethoxy)$_x$(2,2,2-triflurorethoxy)$_y$phosphazene cyclotrimers
(x,y) short hand nomenclature

← Fire retardants.

Extracted ion chromatograms, 100 nL droplets





More ethoxylated congeners (5,1), (4,2), and (3,3) lithiate, and hydrate

(4,2) and (3,3) lithiate, form solvent complexes

(2,4) and (1,5) protonate and lithiate, form solvent complexes

The Department of Chemistry
McMicken College of Arts & Sciences

UNIVERSITY OF Cincinnati

Droplet Based Sampling of RNA Hydrolysates by Induction Based Fluidics

Robert Ross[1]; Manasses Jora[1]; Drew Sauter[2]; Andrew Sauter III[2]; Patrick A. Limbach[1]
[1]University of Cincinnati, Cincinnati, OH;
[2]nanoLiter, LLC, Henderson, NV

Overview

The goal of this study was to couple an inductive charging device to a liquid chromatography separation with a focus of lowering the LOD for standard RNA nucleoside analysis. As such, a synthetic test mix comprised of cytidine, uridine, 5-methylcytidine, adenosine and 2'-O-methyladenosine were separated by means of capillary chromatography and delivered into the mass spectrometer by using a modified inductive charging source powered by a modified inductor coupled to a digital programmed energy and polarity pulsed DC source[1].

Introduction

Post-transcriptional chemical covalent modification of adenosine, guanosine, uridine and cytidine occurs frequently in all types of ribonucleic acids (RNAs). In ribosomal RNA (rRNA) and transfer RNA (tRNA) these modifications make important contributions to RNA structure and stability and to the accuracy and efficiency of protein translation. These modifications can be present at very low levels and their analysis can be challenging. This work builds on previous work where the utility of Inductive Based Fluidics (IBF) as a sample introduction method is examined while coupled to an LC platform. Because IBF creates inductively charged droplets instead of an electrospray, theoretically, a droplet sampling method would allow for greater sensitivity as more sample would enter the mass spectrometer.

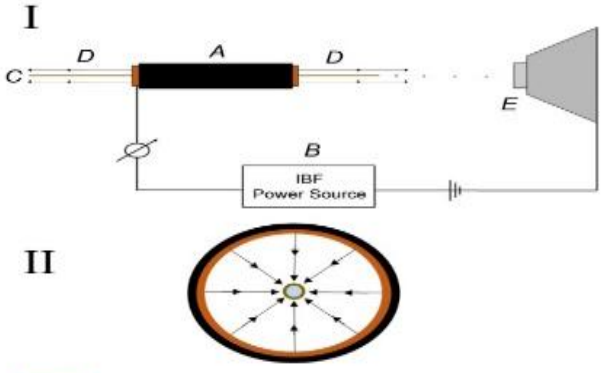


Figure 1. Schematic of inductor employed in this work, inspired by R.W. Kiser[2]
I A: Insulated copper tube (90 x 6 mm) B: Inductive charging device C: Capillary tubing (360 X 50 μm) with flow from column D: Field lines E: Inlet to mass spectrometer.
II Forward view of IBF charging tube showing nexus of field lines onto the sampling capillary

Methods

An equimolar RNA hydrolysate mixture was separated on a porous graphitic carbon packed capillary column inserted into an in-house inductive charging tube with capillary positioned 2-4 mm from inlet orifice. Mass spectra were recorded in positive polarity on a Thermo Fisher LTQ-XL. A capillary temperature of 275 °C, spray voltage of 0 kV, capillary voltage of 0 kV, and tube lens at 0 kV. IBF device was set to – 2000V and pulsed + and - with 2 s intervals over a 40 min acquisition. Data acquisition was through the Thermo Fisher Xcalibur software.

Results and Discussion

Five RNA nucleoside standards, cytidine, uridine, 5-methyluridine, adenosine, and 2'-O-methyladenosine were separated and sampled using the IBF device. Extracted ion chromatograms of the analytes are shown in Figure 2.

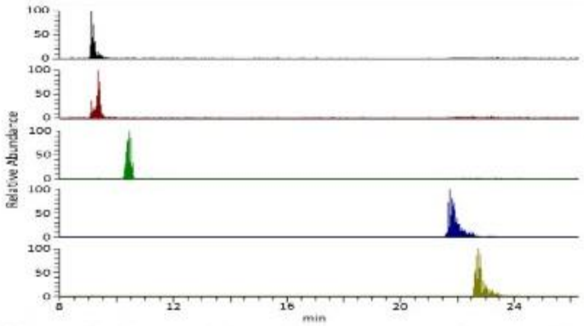


Figure 2. Extracted ion chromatograms of nucleosides cytidine, uridine, 5-methyluridine, adenosine, and 2'-O-methyladenosine separated on a PGC capillary column and introduced into the mass spectrometer by inductive charging.

Droplets were delivered with a 2 s interval over a total run time of 40 min. The total ion chromatogram (TIC) showed steady reproducible droplet peaks throughout the gradient. Each peak in the TIC corresponds to a single droplet delivered via IBF, Figure 3.

XICs of individual nucleosides were generated, with a signal response generated over a single droplet peak in the analyte elution or across the entire set of droplet peaks generated from each analyte. The mass spectrum obtained when summing across the entire acquisition window illustrates one advantage of pulsed operation wherein the background is significantly reduced as illustrated in Figure 4.

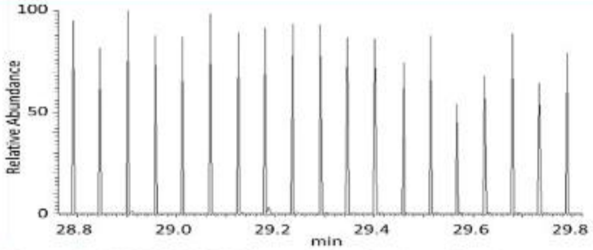


Figure 3. IBF-LC-MS/MS data of droplet introduction over a one minute acquisition window.

The intervals between droplet arrival in the mass analyzer are characterized by no background, which can be reflected in the summed mass spectral data. More Importantly, the ion abundances present within a single droplet are similar to the integrated peak values as previously shown by Groenwold, et al.[3]

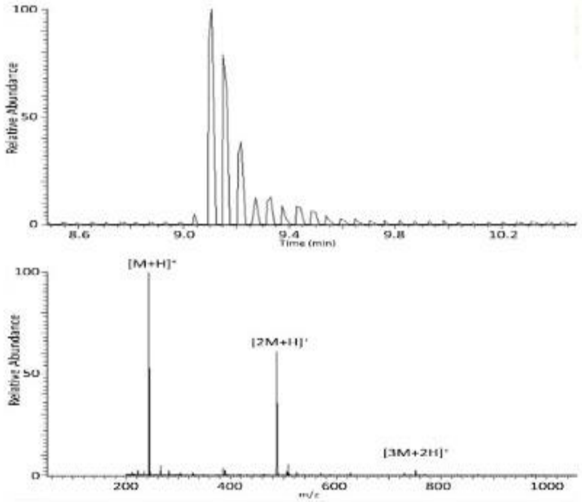


Figure 4. IBF-MS data of the nucleoside cytidine introduced dropwise by inductive charging. (a) Total ion chromatogram showing droplet introduction. (b) Mass spectrum of cytidine obtained by averaging over one peak in the acquisition window.

Droplet desolvation may be more efficient than nESI. This could limit sampling bias for mixtures if ion generation is influenced more by the kinetics of desolvation rather than the thermodynamic partitioning of the analytes with different hydrophobicities at the droplet surface. Figure 5 shows XICs of the equimolar nucleoside mixture with the relative abundances listed. This data aligns with previous work[4] suggesting that kinetics may better represent ion generation with droplet sampling. More experiments are planned to strengthen this argument

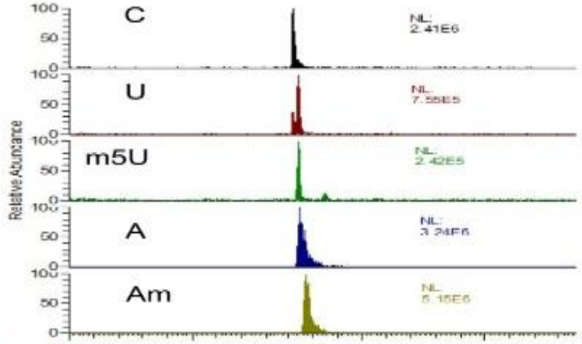


Figure 5. Mass spectra of nucleosides (5 ng/μl), cytidine, uridine, 5-methyluridine, adenosine, and 2'-O-methyladenosine. Droplet introduction could minimizes the sampling bias related to nucleobase hydrophobicity

Conclusions

A programmable IBF droplet source appears suited for nucleoside UPLC sample introduction and mass spectrometric analysis. Preliminary results show this droplet based approach is equivalent to or may exceed nESI. Work to determine LOD's and more is continuing.

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Acknowledgments

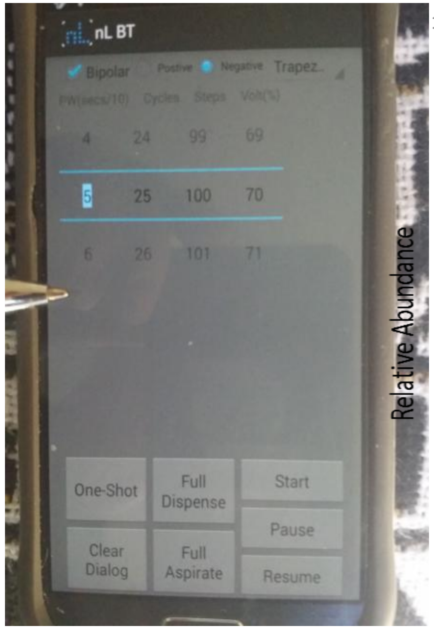
The presenting author would like to thank the members of the Limbach Research Group for their assistance. Financial support of this work was provided by the National Science Foundation (CHE1212625). The nanoLiter Programmable Wave device was acquired under license from nanoLiter LLC

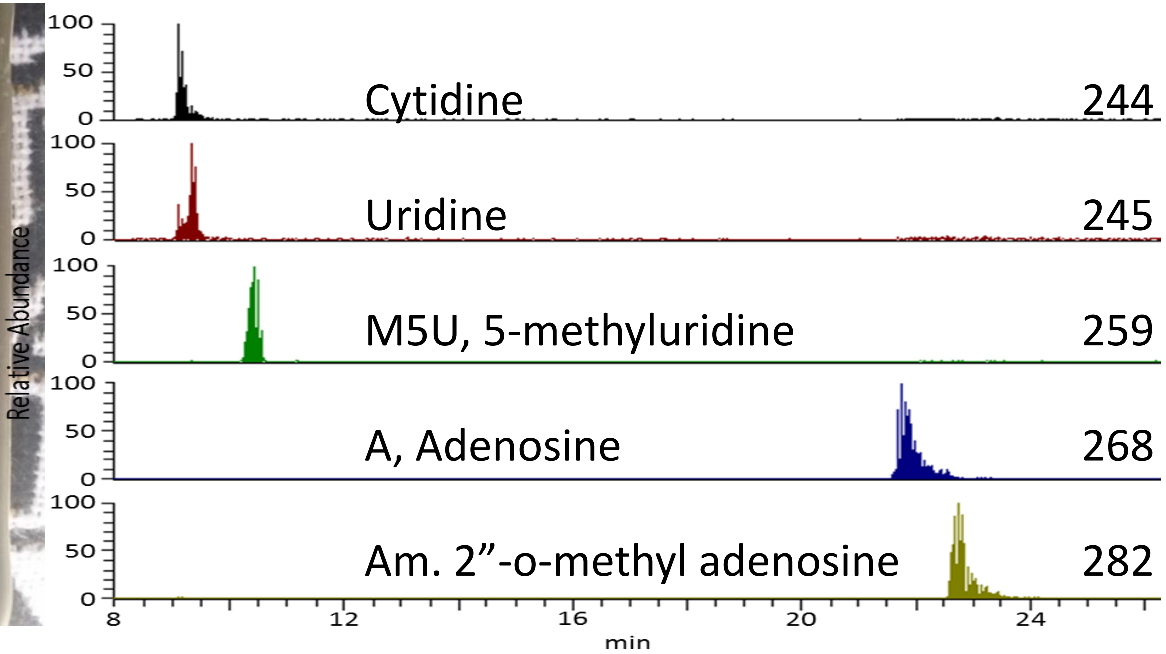
100% INPUT EFFICIENT ESI UPLC MS. ANDROID CONTROLLED,

ALLOWS YOU TO CREATE & CONTROL DROPLET ENERGY, IT'S WAVE ENERGY & VOLUME, POLARITY & LOCALE.

Equimolar 5 pg/nL, spray, cap, tube v= 0, 2 sec, 40 min, PGC capillary 10 nL/sec, cap temp = 275 C, m/z range 50- 500?. LTQ-XL.

XIC UPLC MS +eV 5 nucleosides (M + H)+





Extracted ion chromatograms of nucleosides, top, cytidine, uridine, 5-methyluridine, adenosine, and 2'-O-methyladenosine separated on a PGC capillary column and introduced into the MS by inductive charging. per Asilomar 2016 at U. of Cincinnati with Drs. Ross & Limbach. Also, see P. Limbach, et al , JMS Oct. 2015.

THE PROGRAMMABLE WAVE ALSO MAKES EXCELLENT MALDI, SIMS, LDI CRYSTALS VIA THE ***SAME IBF DEVICE!***
IT ALSO HAS WET LAB APPLICATIONS, e.g., TLC, and OTHER VERY COOL APPS (OVER).

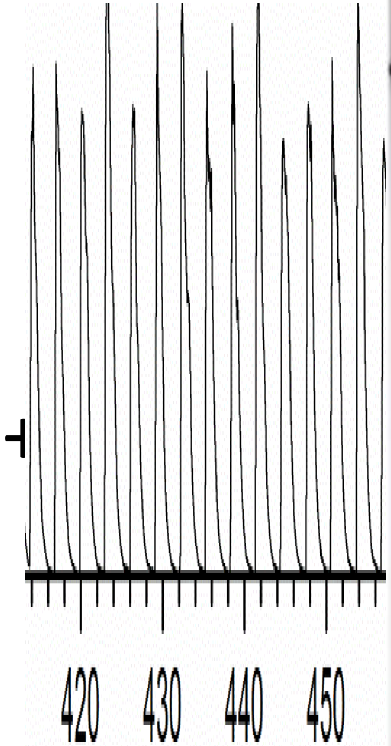
Drew Sauter, nanoLiter LLC, 217 Garfield Drive, Henderson, NV, 89074, USA, nanoliter,com, adsauterjr@gmail.com.

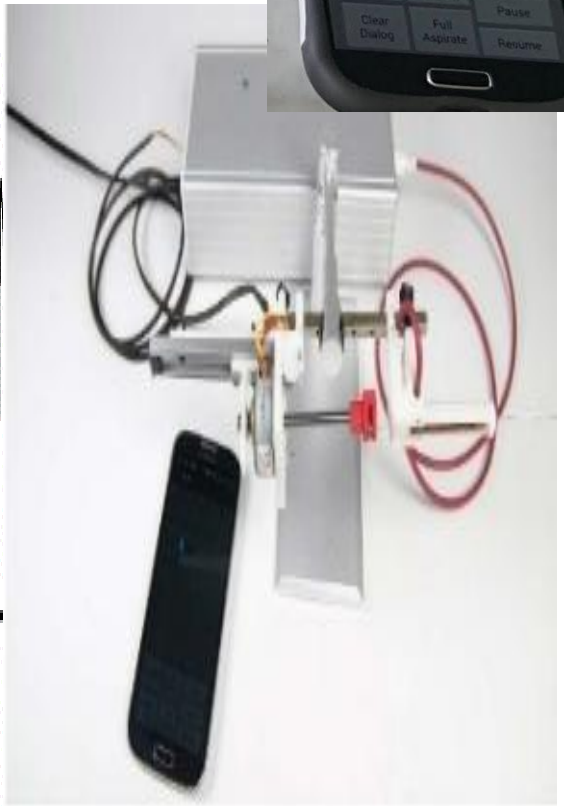
CAN IBF "ESI" MS INCREASE SENSITVITY BY 4 to 6 ORDERS OF MAGNITUDE ?

1. Shooting **all of the sample *inductively*** into the ion inlet tube, gets, ca. 100-1000x more moles into the MS.

2. Also, **pulsing** the energy can **get ca. 100 to 1000x** in moles/time into the ion inlet tube!

3. With **PERFECT DESOLVATION** 4 to 6 orders of magnitude sensitivity increase may be possible via our IP.





Digital bipolar 3 second shots. Bruker u-ToF. INL

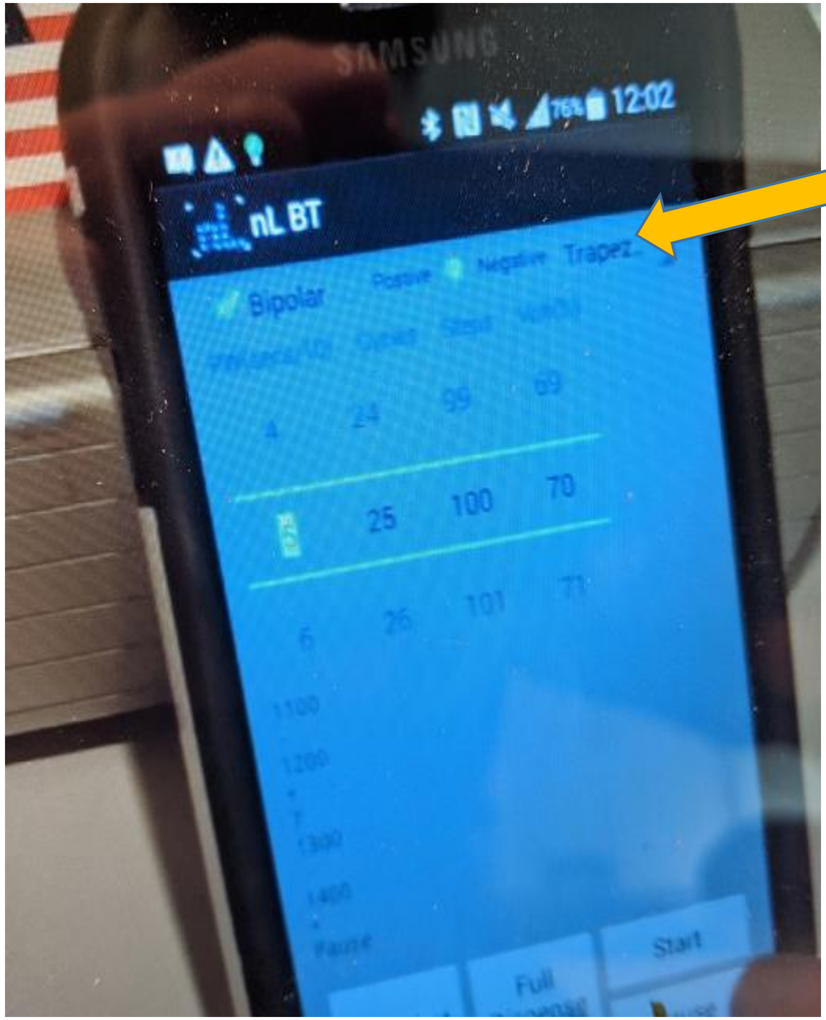
(See references)

Android Cell Phone Energy Programmer for Simplicity.

Increase In ESI Sensitivity of 4-6 Order of Magnitude for MS of Cells + Optimized Desolvation Conditions?

.

The Best Inductive Energy Program & Other Conditions Will Yield Highest Sensitivity , (i.e., I/P or I/w).
More Observations/Data Coming After Covid-19. Seeking Collaborators.



Energy Functions vs. Time.
Four selectable wave functions

Other variables include

- Position
- Energy
- Polarity,
- Mode thereof

Acquisition Parameters

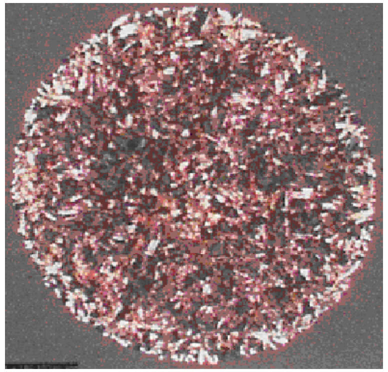
- PW,
- Cycles,
- Steps,
- Applied Energy

Also

- Solvents, Flow, Temp, arrangements, etc.

VIDEO CLIPS/PICS

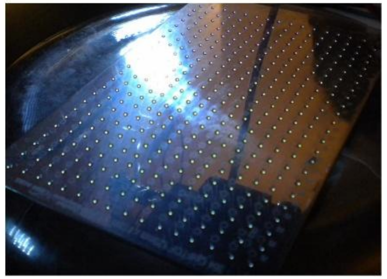
INDUCTION BASED FLUIDICS FOR ESI, MALDI, SIMS, LDI and Dispensing apps.



IBF, shooting a droplet up!

IBF shooting a droplet into a TOF

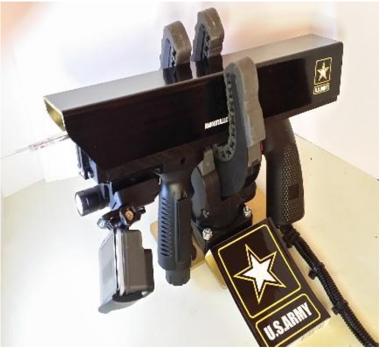
IBF Shooting droplets down w/ Spark Hollland robot!



IBF Shooting 100% of droplets into a TOF, slow at 5sec/drop, Groenewold, G. , INL, AC June 2013.

IBF Shooting 100% of droplets into a TOF, fast, ca. 2 Hz.

IBF 384 parallel 250 nL dispense using normal tips.



IBF MALDI Gross JASMS Calibration Curve.

Android MALDI Dispenser and ESI Source.

Pure IBF research, Professor Scheeline, U of Illinois, AC Jan 2014.

Miscellaneous Old/New Video Clips

SUMMARY

Electric fields, powerful MS tools for ESI & MALDI, increased sensitivity! 4 to 6 orders of magnitude?

Major sensitivity increase (> 10x) result published/presented for MALDI, SIMS, (LDI?), ESI (Infusion and UPLC MS).

Accurate **nL dispensing** for production of crystals, more.

Parallel **Millisecond *Robotic*** Dispensing.

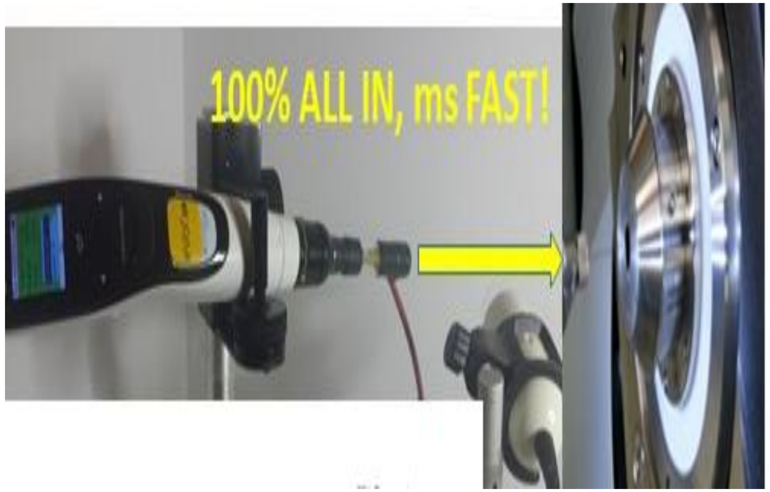
Dispense nLs **viscous liquids**, polymers, whole blood, more.

Manufacture of **electrets**.

"Anything" can be made w/E field to **MORPH** capillaries, !
Pipette Tips, Syringes & Pumps,
Pipettes, Chips, LC, your devices into IBF source!

IBF VERY ECONOMICAL COMPARED TO OTHER TECHS !

SIMPLE TECH, *INK JET LIKE. OCCAM'S RAZOR!*



http://www.nanoliter.com/2020asmsweb.pdf

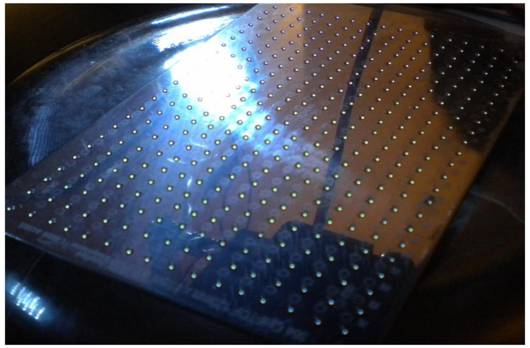
"Unbiased" Ambient Comparison

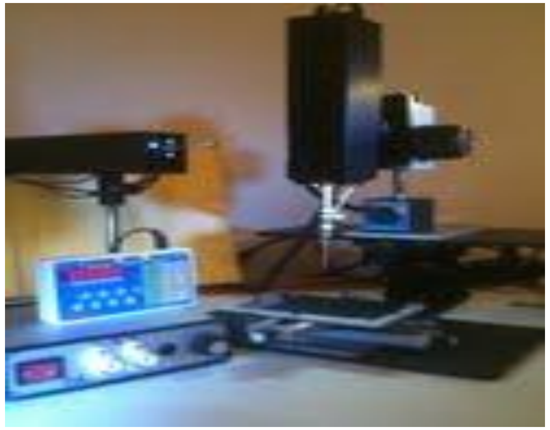
	MALDI	SIMS	LDI	LC/MALDI.	ESI INFUSION MS	ESI UPLC MS	nL ms dep	$
IBF	P	P	(?)	Pr	P	Pr	P	Lowest
Parallel Sprayer	No	No	No	No	?Y	?Y	No	?
DART	No	No	No	No	Yes	No	?	Med
DESI	**No**	**No**	**No**	**No**	**?**	**No**	**No**	**Med**
Acoustic	No	No	No	No	Claimed Practicality/Cost Questioned by me!	No	?	Very High!

Seeking Interested Corporations, Gov't Agencies and U's.

For

Analysis of Cells/Organelles, Native MS, Actinides, Instrumentation, Commercialize?





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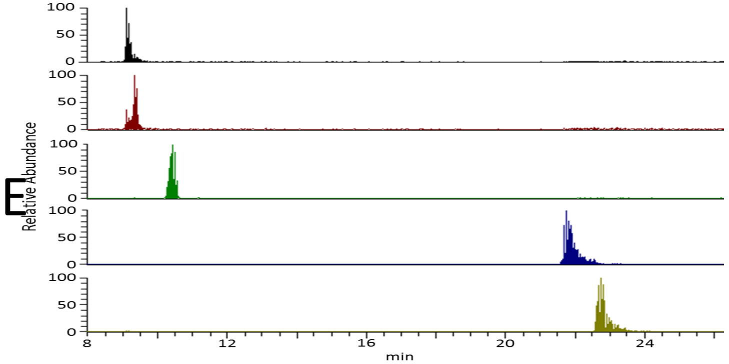
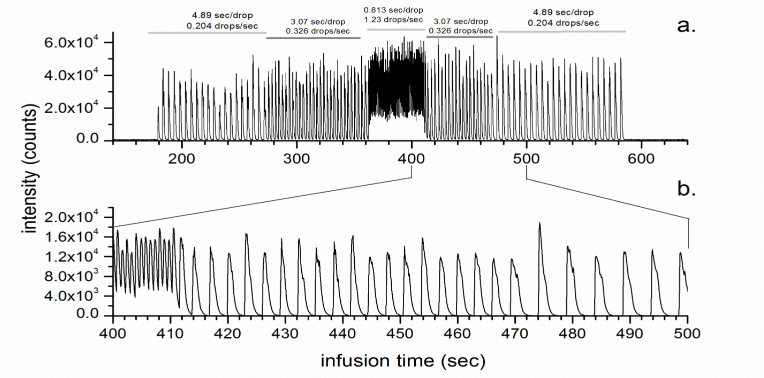
702-882-5413





ABOUT

Working with many over the last 50 years (See links.), we changed GC - MS in the USA, directed early ICPMS funding to DOE worked with Extrel Willoughby) on very early LCMS that was purchased by Waters and did the earliest QQQ GC MS MS and LCMSMS. Now, we've done rapid, 100% "ALL IN" ESI droplet infusion & UPLC MS (ASMS 17/18) as O ur Android source RAPIDLY flys droplets into MS's or onto targets per ASMS 2020.

CLIENTS/COLLABORATORS: U's of Ill, WI, CA, Cinn., MUSC, Wash. U. St.L, UCSD, USF, USU, US Army APG, ECBC, Dugway PG and Natick, Abbott , Biogen Idec, Genentech, Amgen, Hitachi, Allergan, Merck, Sciex, Spark, Douglas, NIH, NIST, USDOE INL, Ga Tech, UNH, Duquesne, SIS, Adaptas, Caltech, USDA, Leonardo and Los Alamos National Lab, ORNL, RTI, DOJ in DC, Hanford and MRI.

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