100% Input Efficient ESI UPLC MS Sample Introduction and MALDI, SIMS, LDI Sample Placement Via An Inductive Approach. Universal?

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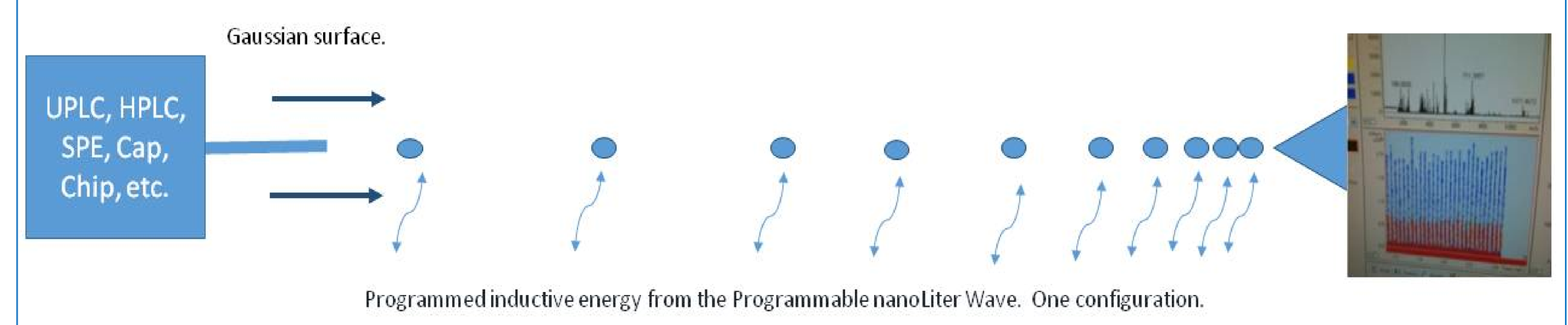
Introduction

We've invented and patented a technology called induction based fluidics(IBF) over the last 15 years (1-22). As qualitative and quantitative MS analysis are simply different parts of the same equation (23), whatever the application, this core principal of mass spectrometry applies to ESI and MALDI applications be it analysis for protein therapeutics analysis, inorganic determinations or applications like marijuana analysis or analysis of battery fluids.

New techniques that improve analysis speed and sensitivity and that allow one to arrive at answers faster and with higher confidence are in and of themselves of value properly vetted in the peer review literature, See http://nanoliter.com/references2015.pdf.

Here we report how induction based fluidics (IBF) has been used to increase analysis speed, sensitivity of MS analysis pictorially and in detail by MALDI and ESI for proteins and peptides, oligonucleotides, metals (Lanthanides and Actinides), explosives and drugs of abuse, and other analyses using IBF. as we discuss the attributes of droplet and related analysis.

IBF is analogous to printing into an ESI. Or shooting the drop down to a surface produces excellent MALDI crystals.



Programmed inductive energy from the Programmable nanoLiter Wave. One configuration.

Example Ink Jet Calculations. IBF is NOT an Ink Jet, but it's analogous.

Below we ignore all but electrical and drag forces as they are the largest forces inside printers.

F = ma = Fe + Fd

mdu(z)/dt = qE(t) – Ku

Uz = qE/K(1-exp-t/tm)) = velocity

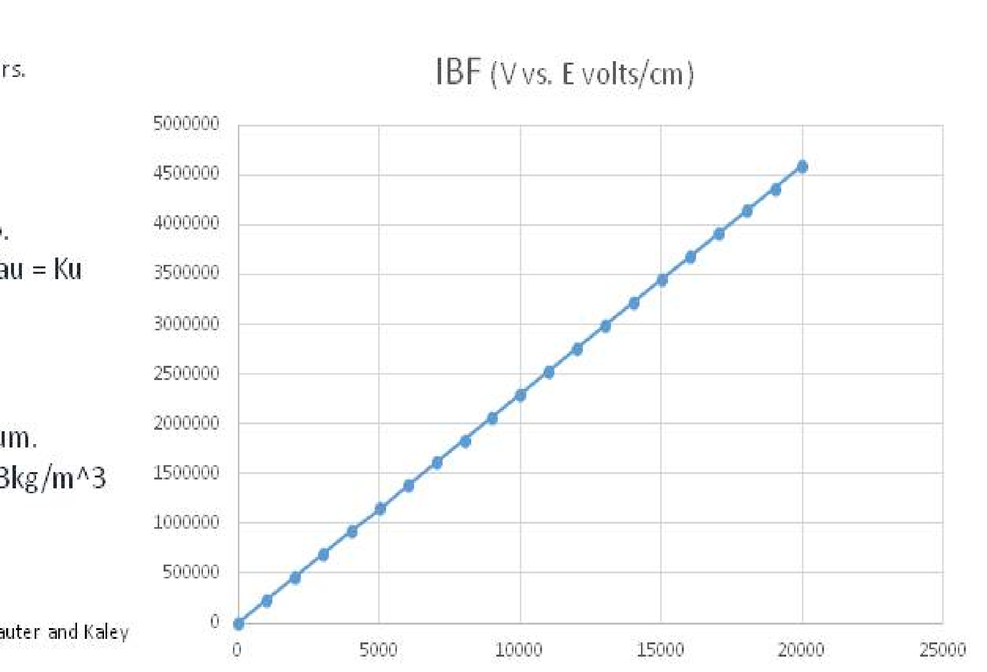
Z = qEo/K(t-tm(1-exp-t/tm)) = displacement

tm = m/K = m/(6pina) = (2/9)(a²/n) = 2.5 ms

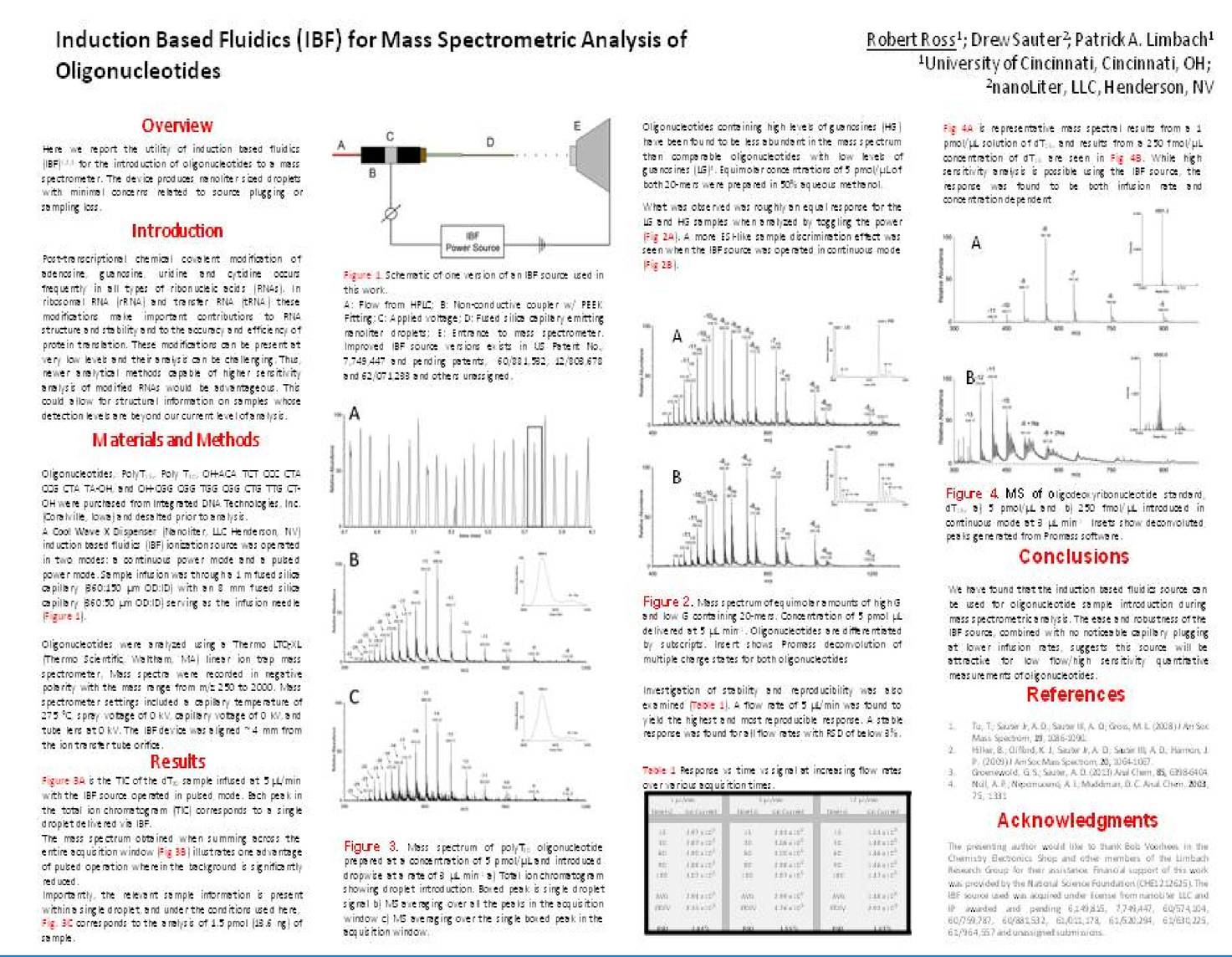
Zm = ((q/m)E²/2) = 16 mm

Fe = external field, qEo.
Fd = drag force = 6pinau = Ku
U = velocity

n = dynamic viscosity.
a = drop radius ca. 15 um.
a = density of ink, 10³ kg/m³

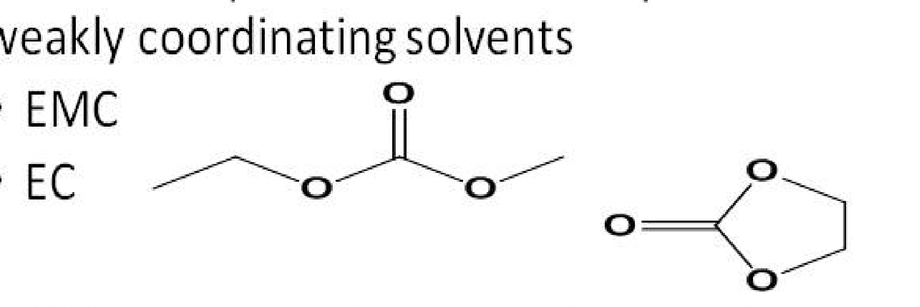


IBF of Oligonucleotides via Pulsed Energy. Droplets appear to desolvate better than sprays,(21)!



Single droplet analysis of battery electrolyte.

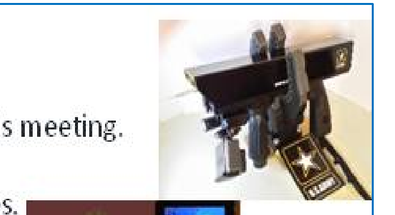
- Coin cells: dependent on Li^+ transport in carbonate electrolyte – weakly coordinating solvents
  - EMC
  - EC



- Breakdown can lead to H_2 production, flammability issues
- Diagnostic electrolyte analysis needed
  - Extremely limited sample volumes (a few μL)

IBF MS analyses were conducted using a Bruker MicroTOF-QII, modified by the removal of the ESI source. A Hamilton 10 μL syringe equipped with a fused silica needle was used in the IBF source. The needle was positioned approximately 0.3 cm from the aperture of the ion transfer capillary on the instrument, which was operated in the positive ion mode, using the standard "Tune Low" method.

IBF User Successes.



IBF is being used by the US Army for classified agent dispensing projects and MS R&D w/ optional GoPro camera, J. Olyer, et al poster this meeting.

IBF being used for MS Analysis of Oligonucleotides. NEW!!! JMS paper w/ U of Cincinnati yields nanomolar sensitivity for oligonucleotides.

US Department of Energy is using IBF in the field to analyze radio-active elements at fg levels ……WITHOUT an ICP !

IBF is being used to introduce samples into a MS from an OPERATING battery at a National Lab.

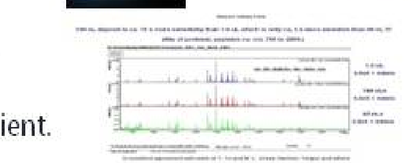
USF, NIH, NIST & JEOL., have publish that by using nLs for MALDI, SIMS, (LDI?) & DART that MS sensitivity increases by 10,20-100x LITERALLY!

University of Wisconsin has used IBF for single cell MALDI identifying six new ocular proteins. We shot cells into an ESI at gov't lab.

University of Illinois published that IBF can fly nanoLiters of liquids into levitated microliters to study wall-less reaction kinetics.

For Abbott, nanoLiter LLC used IBF to dispenses PVA, w/ave. MW of 300,000, in pseudo 3D "printing." app.

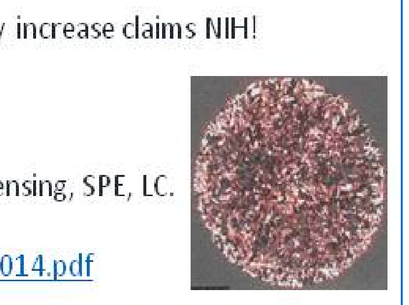
At Genentech, an nL wave demonstrates 20 x improvement in MALDI sensitivity for proteins, peptides.



USF used IBF to make electrets. LC/MALDI for Sciex who offered to license! LC ESIMS for commercial client.

NIH, in it's first application of IBF, PTM's of tublin (glycosylation) were first ided, in actual brain cancer samples given a 100x sensitivity increase claims NIH!

Sciex offred to license IBF for ESI LCMS and for LC/MALDI in 2006. Parallel 8 channel IBF LC demoed with dyes.



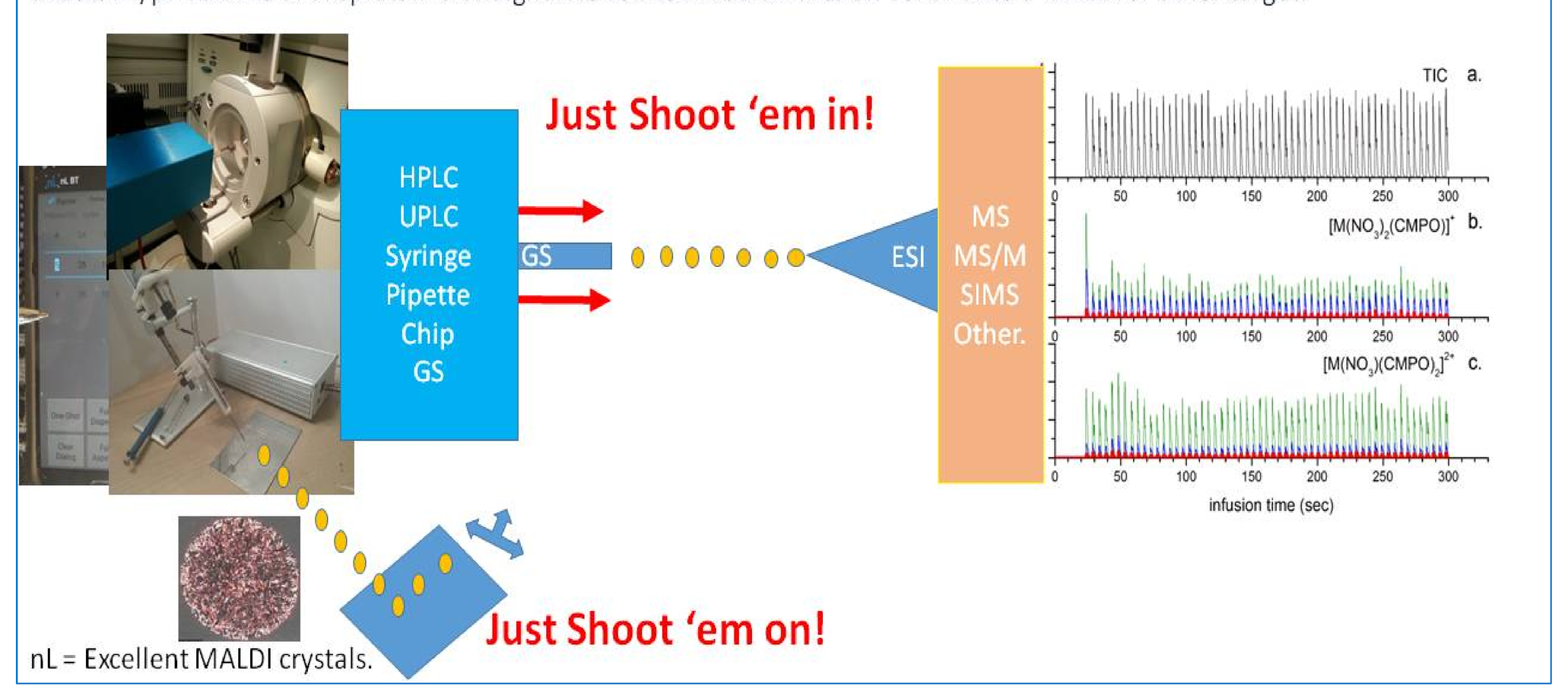
nanoLiter morphs Roche polypipettor for Douglas and Spark Holland's systems into parallel 384 or single channel millisecond nL dispensing, SPE, LC.

See more here. http://www.nanoliter.com/nanoliterhasdone121213ver3.pdf & some references, http://nanoliter.com/references2014.pdf

Example users: U's of Ill, WI, CA, Cinn., MUSC, Wash. U., USF, USU, US Army APG, ECBC and Natick, Abbott, Biogen Idec, Genentech, Amgen, Hitachi, Allergan, Spark, Douglas, NIH, NIST, USDOE INL, Ga Tech, UNH, Duquesne, NASA, Air Force, Air Force, and Sciex offered to license. +

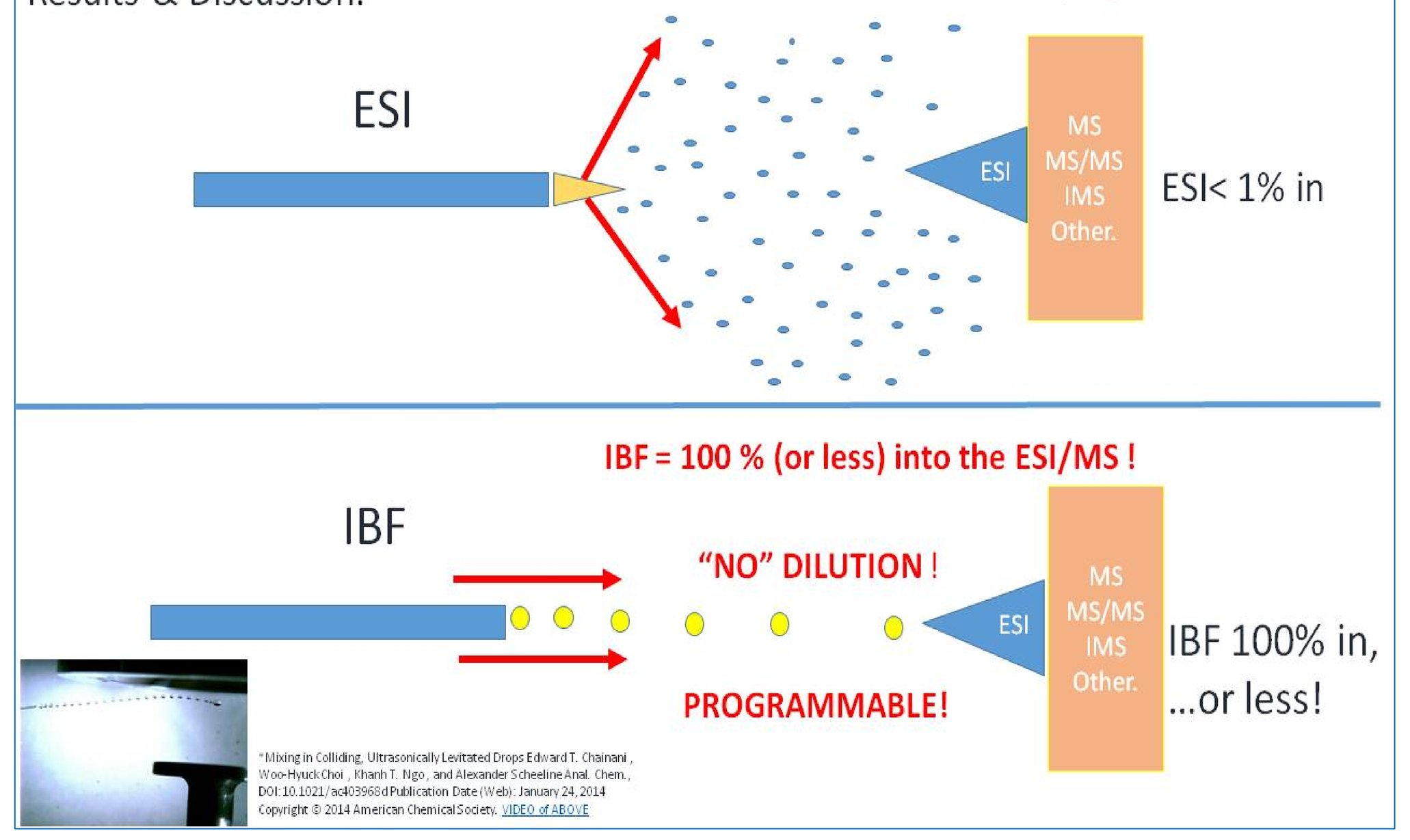
ONTO or INTO via E INDUCTION to GAUSSSIAN SURFACE,+.

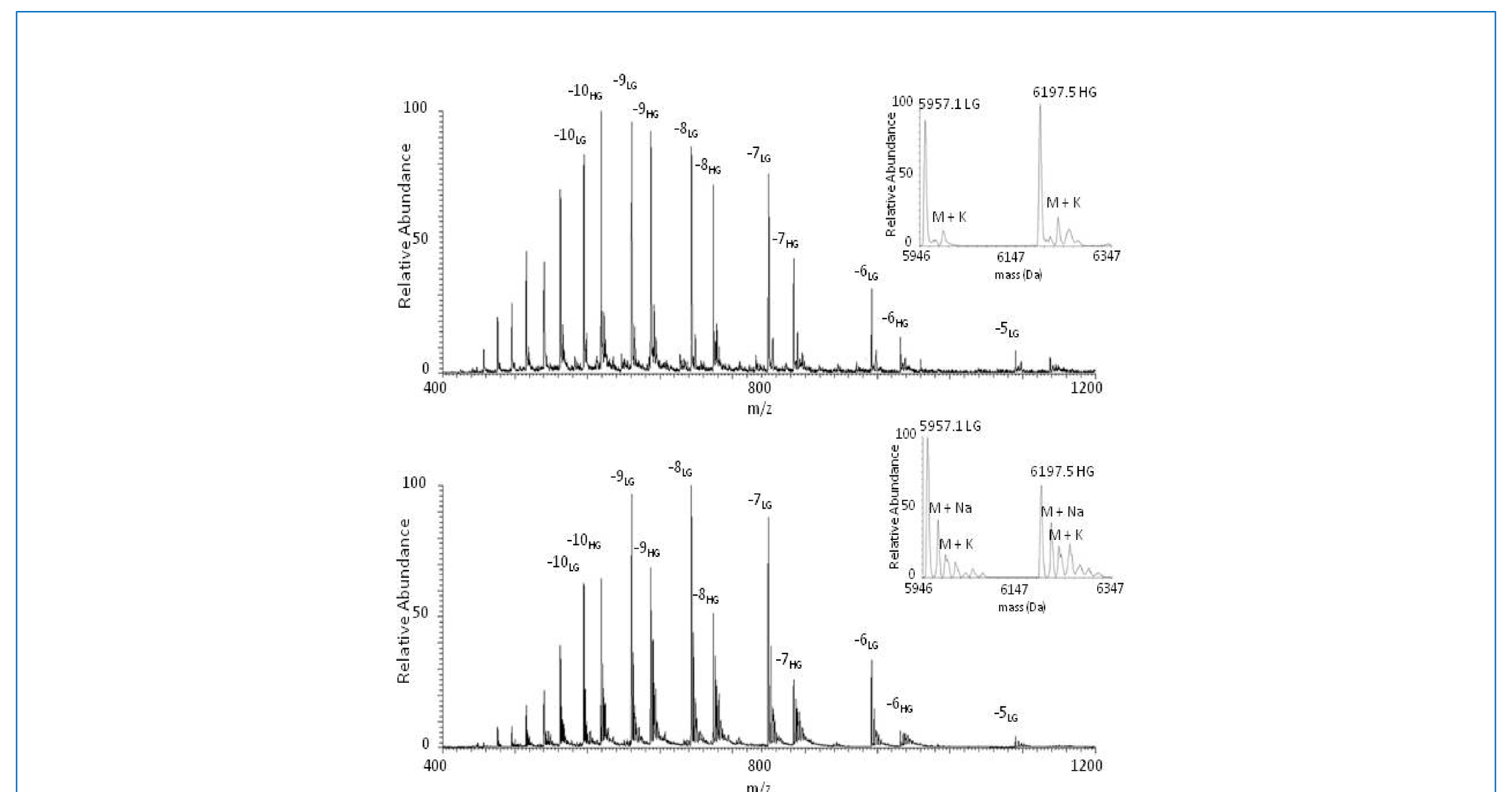
Over the last few years we described induction based fluidics (IBF) in the literature and at meetings as a way to accomplish various tasks in mass spectroscopy, fluidics and sample handling using capillaries, syringes, pipettes, pumps, chips and various LC configurations and other Gaussian surfaces. Specifically, IBF represents one way to make excellent high sensitivity crystals for MALDI ***OR*** to shoot 100% of liquid samples into existing ESI sources generating ESI mass spectra. Essentially, IBF inductively shoots nL/pL volume of droplets in a straight line as shown below into an ESI or onto a MALDI or other target.



nL = Excellent MALDI crystals.

Results & Discussion.

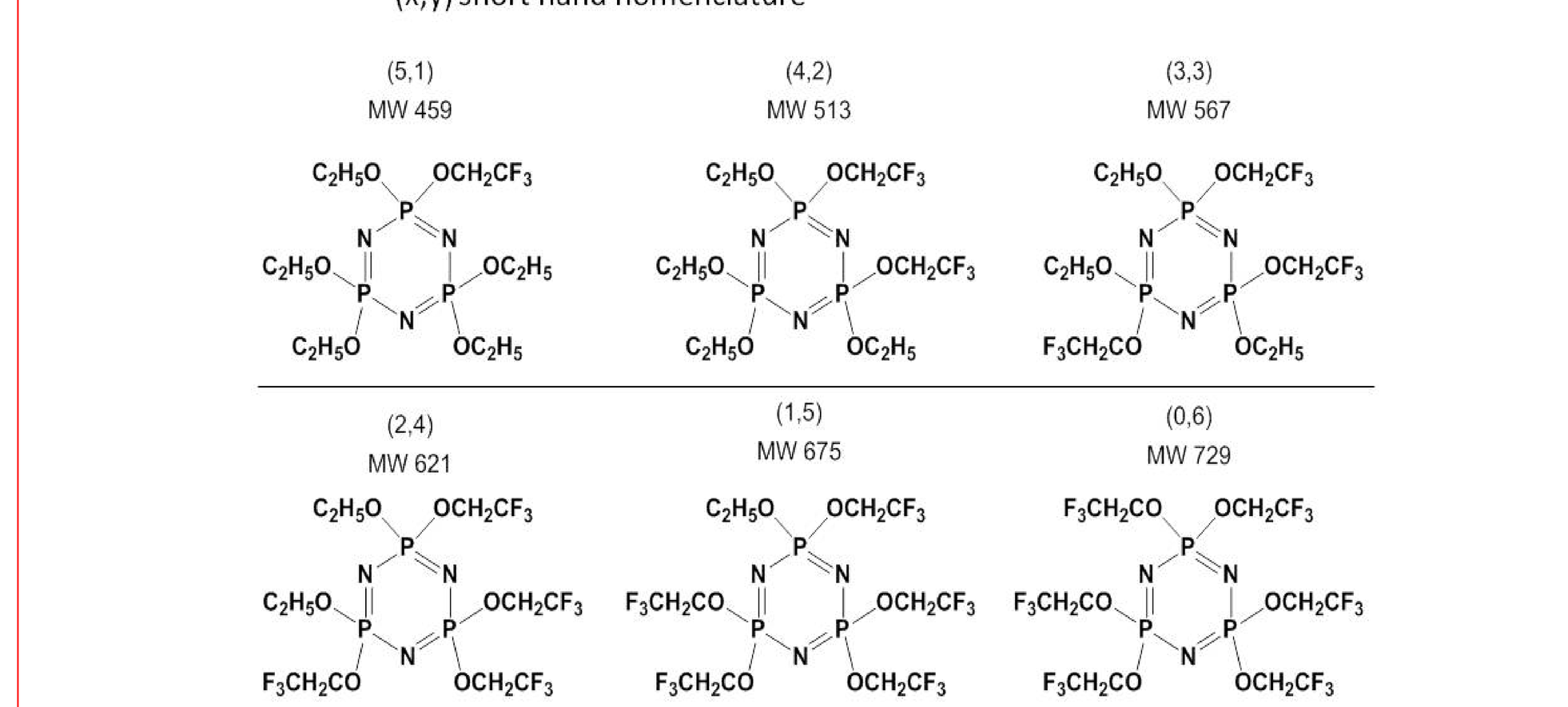




Mass spectra obtained from equimolar amounts (5 pmol/μl) of oligonucleotides 20-mer-LG (5'-ACA TCT CCC CTA CCG CTA TA-3') and 20-mer-HG (5'-CGG CGG TGG CGG CTG TTG CT-3'). Sample introduction in (a) droplet and (b) continuous mode at 5 μl/min. Inset shows deconvoluted molecular ions generated from ProMass 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.

Li⁺ ion battery fire retardant

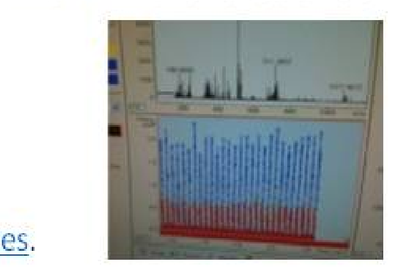
- (Ethoxy)ₓ(2,2,2-trifluorethoxy)ᵧphosphazene cyclotrimers
  - (x,y) short hand nomenclature



Summary

E Induction + "ANY" Gaussian Surface = Source for ESI that's a MALDI Dispenser.

E fields inductively applied to gaussian surfaces = new attributes for old & new fluidic tools!



One IBF device was used for MALDI deposition and 100% ESI sample introduction in, as fast as, ms.

For MALDI, SIMS (& LDI ?) sensitivity is increased by > 10x using nLs compared to uLs for ESI w/IBF, as published.

IBF yields **standard ESI mass spectra**, with major increases in sensitivity, reproducibility for MALDI & ESI. References.

Droplets shot in a straight line appear to **desolvate better** than sprays. Hence, IBF yields at least nanomolar sensitivity for the analysis of oligonucleotides, and maybe better, 21!

IBF droplets can be used to shoot cellular liquids, cells directly into a ESI! USA's best mass spectroscopist:" exciting!"

IBF is being used (USDOE) to MS analyze Lanthanides, Actinides, at **fg levels**, **in the field** , **WITHOUT** a plasma! **6400 determinations** in two days!

IBF is being used by the US Army for safer handling of nLs of chemical agent simulants and agents and for MS.

IBF is used to morph syringes, pipettes, pumps, SPE, chips , LC's into non-touch nL dispensers, even ***pipette/MS***.

Droplets are being shot from an **OPERATING** battery directly into a mass spectrometer by USDOE! New tool!

The application of programmed IBF waves increases the physical precision of droplet dispensing. Work ongoing.

Experimental

In all experiments given here an analogue or programmable inductive energy source was employed to energize a fluid in a Gaussian surface.

The gaussian surfaces could be a capillary, a syringe, a pipette, a chip, an LC or UPLC columns or related device.

Typically a pneumatic flow was inductively energized in a programmed manner, to fly the liquid to a target in the nL/sec flow rate.

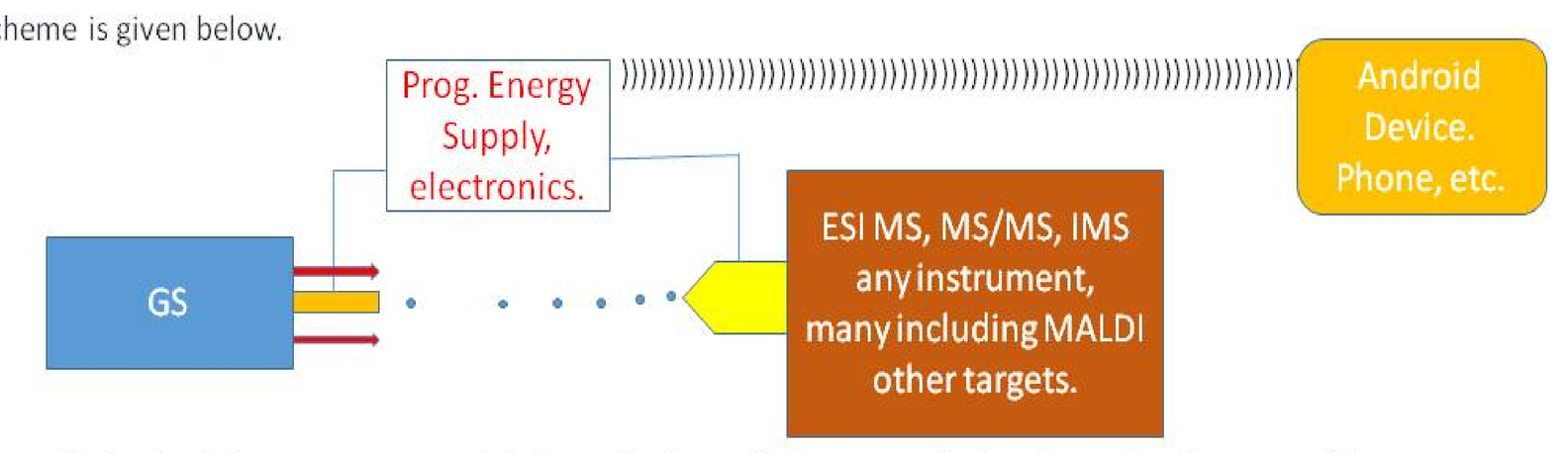
Circuitry was such that a drop or droplet flow was pushed or pulled to a target via appropriate connections, and optionally programmed WAVES.

Targets included MALDI plates, ESI sources or other targets from humans to inanimate objective to scientific instruments.

Fluids can be liquids of all types including whole human blood, polymers, lipids, peptides, proteins, oligonucleotides, etc.

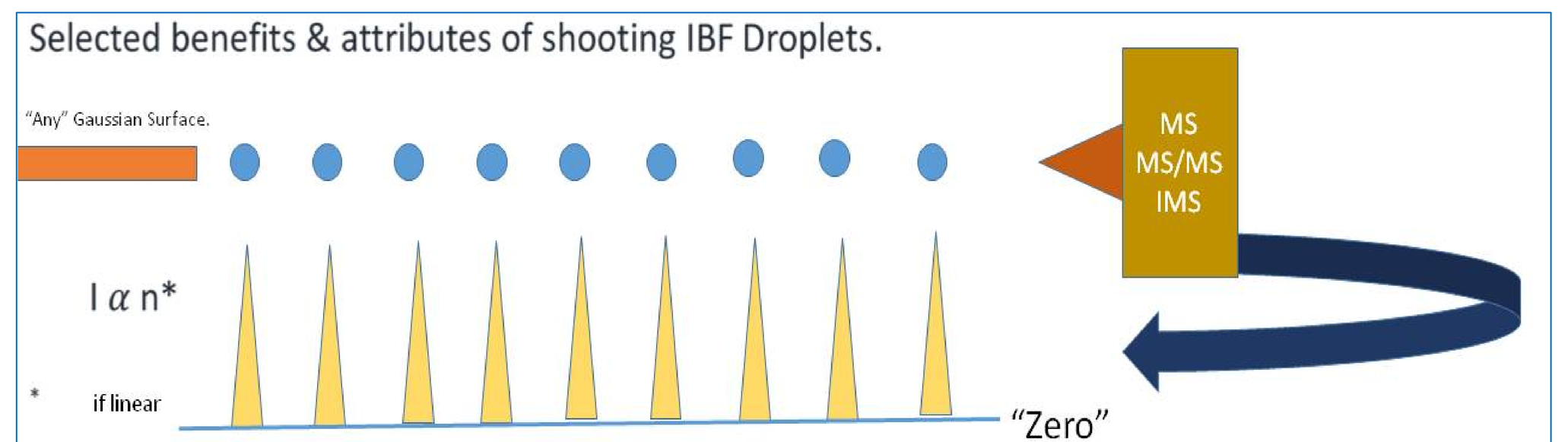
Electric fields applied to said fluids ***in a programmed manner*** with appropriate circuitry to effect ***rapid, directed*** sample placement.

A general scheme is given below.



Note, droplets can programmed to be pushed or pulled as connected and energized in appropriate waves.

Selected benefits & attributes of shooting IBF Droplets.



Droplets shot into an ESI at ca. 1 m/s yield excellent SENSITIVITY because….

100% introduction efficiency,

Signal compression, (fast drops, more moles in less time) dn/dt > a diluting spray.)

High reproducibility, i.e., precise dn/dt, yields highly reproducibility di/dt. Like Printing.

Signal from a "zero" background.

ATTRIBUTES of IBF Droplets.

Programmable

Non-touch, directed dispense over a distance.

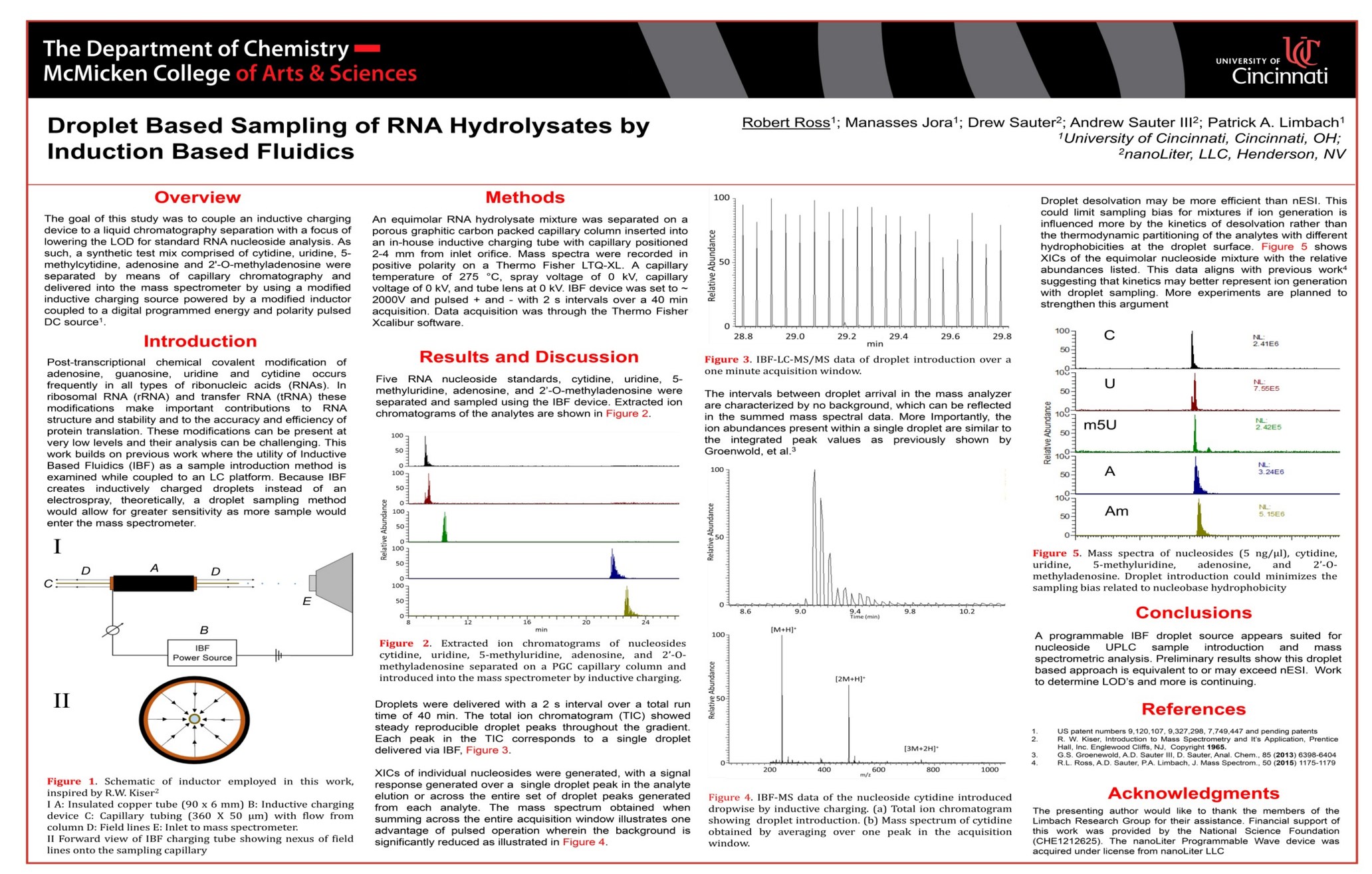
Highly Parallel Dispensing

Viscous liquid dispensing.

Multiple dispensing devices, gaussian surfaces, can be used.

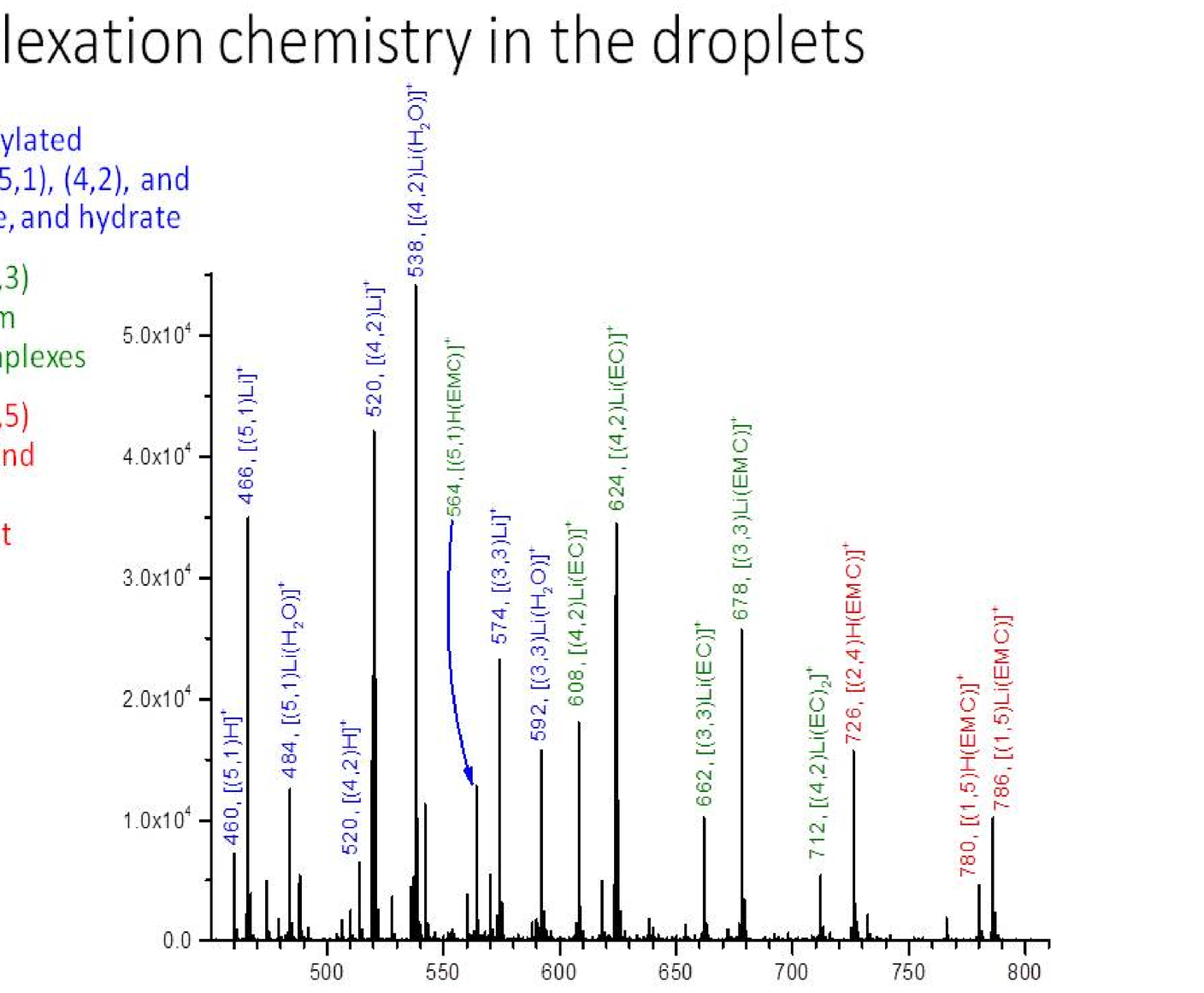
Millisecond dispenses. nL, uL and pL volumes w/varied rate

Droplets appear to desolvate better than sprays increasing the sensitivity of even oligonucleotides(21).



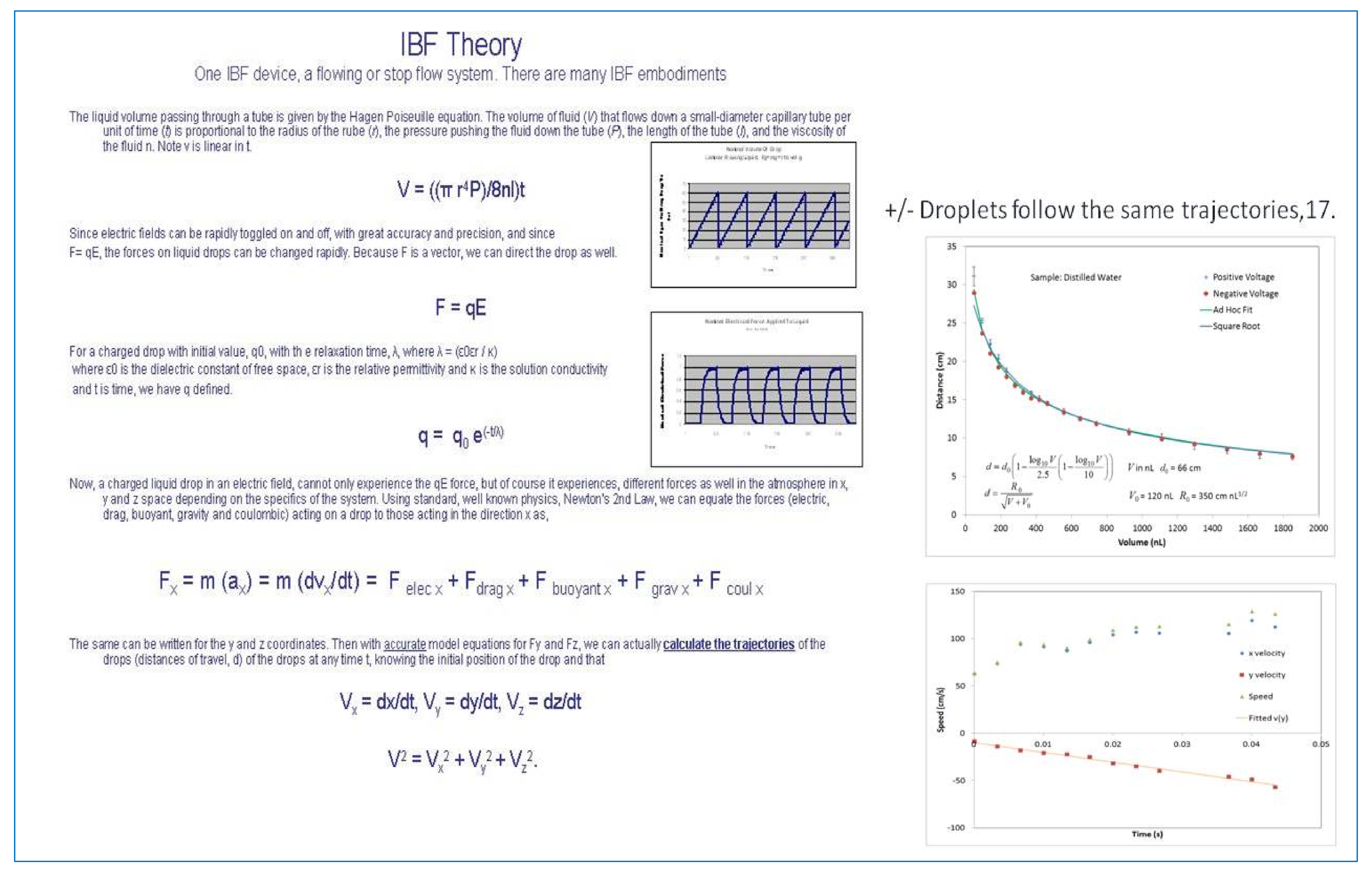
Complexation chemistry in the droplets

- 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

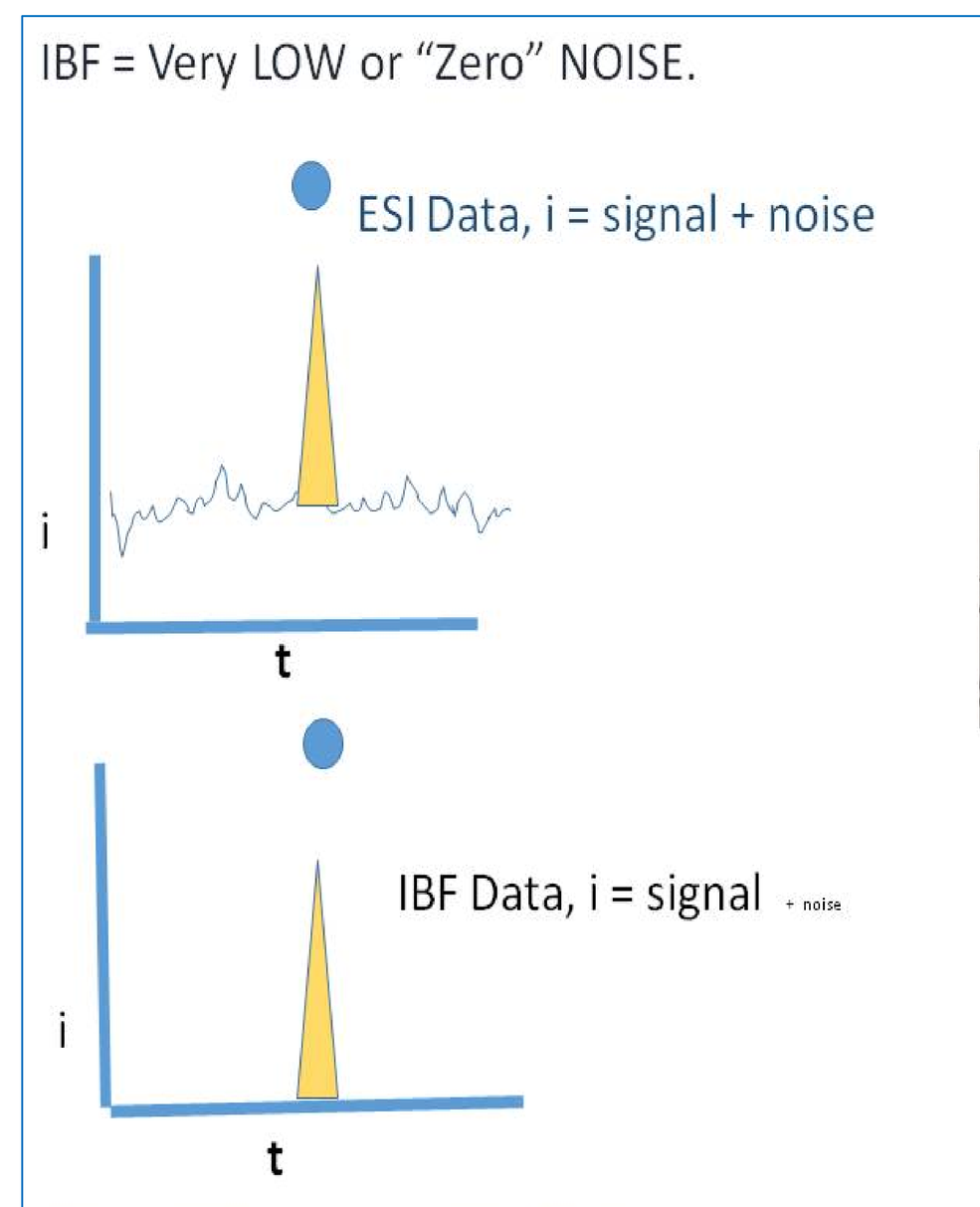


IBF Theory

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

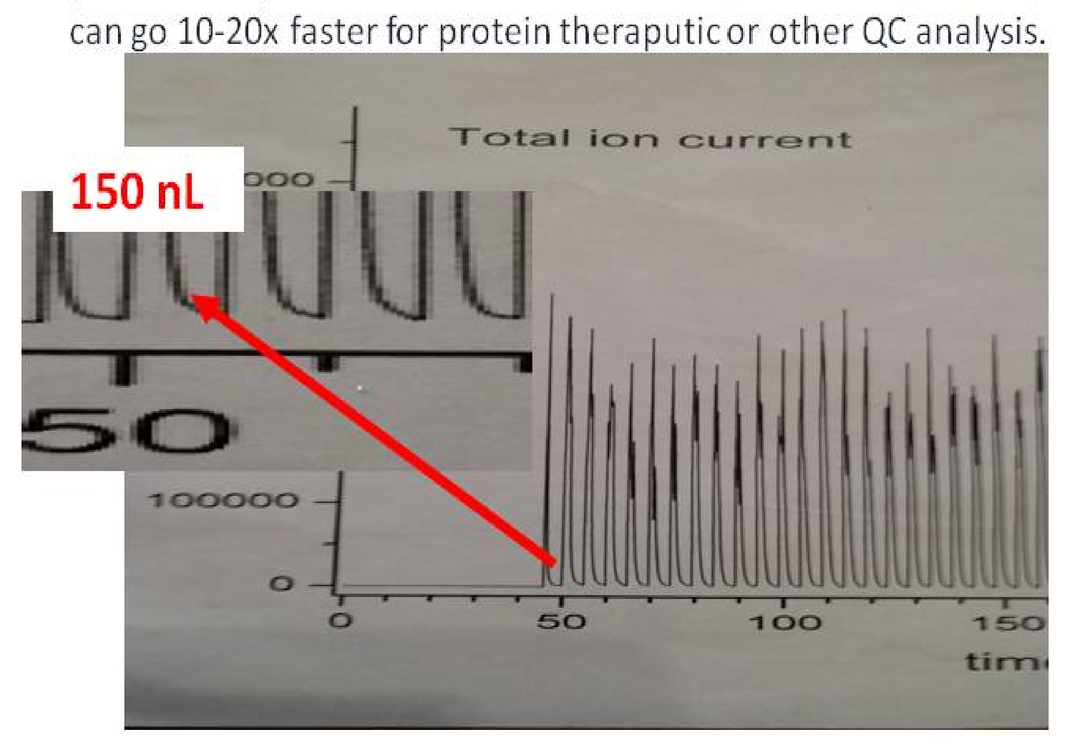


IBF = Very LOW or "Zero" NOISE.



IBF= Beautifully sharp peaks! i.e., dn/dt, > Spray.

Cell Phone pic of droplet MS Data. Note: 1. reproducible peak shape, 2.excellent peak symmetry with 3. rapid clear out time (ca. < 3 sec.) and with 4. low dead volume, here 5 sec/150 nLs, can go 10-20x faster for protein therapeutic or other QC analysis.



Bruker micro tech., w/ nanoLiter Cool Wave, droplet dispenser/ 3sec at flow ca. 70 nL/sec. Sample 10 Hz at ca. 150-1500 amu. TIC CMPO acquisition, (16).

IBF = 100% into the ESI. **PLUS,** it does so from the lowest background @ the fastest dn/dt rate.

IBF Wave type & speed impacts the precision of dispensing and MS introduction reproducibility.

Droplets shot onto a non-conductor from the nL Programmable Wave.

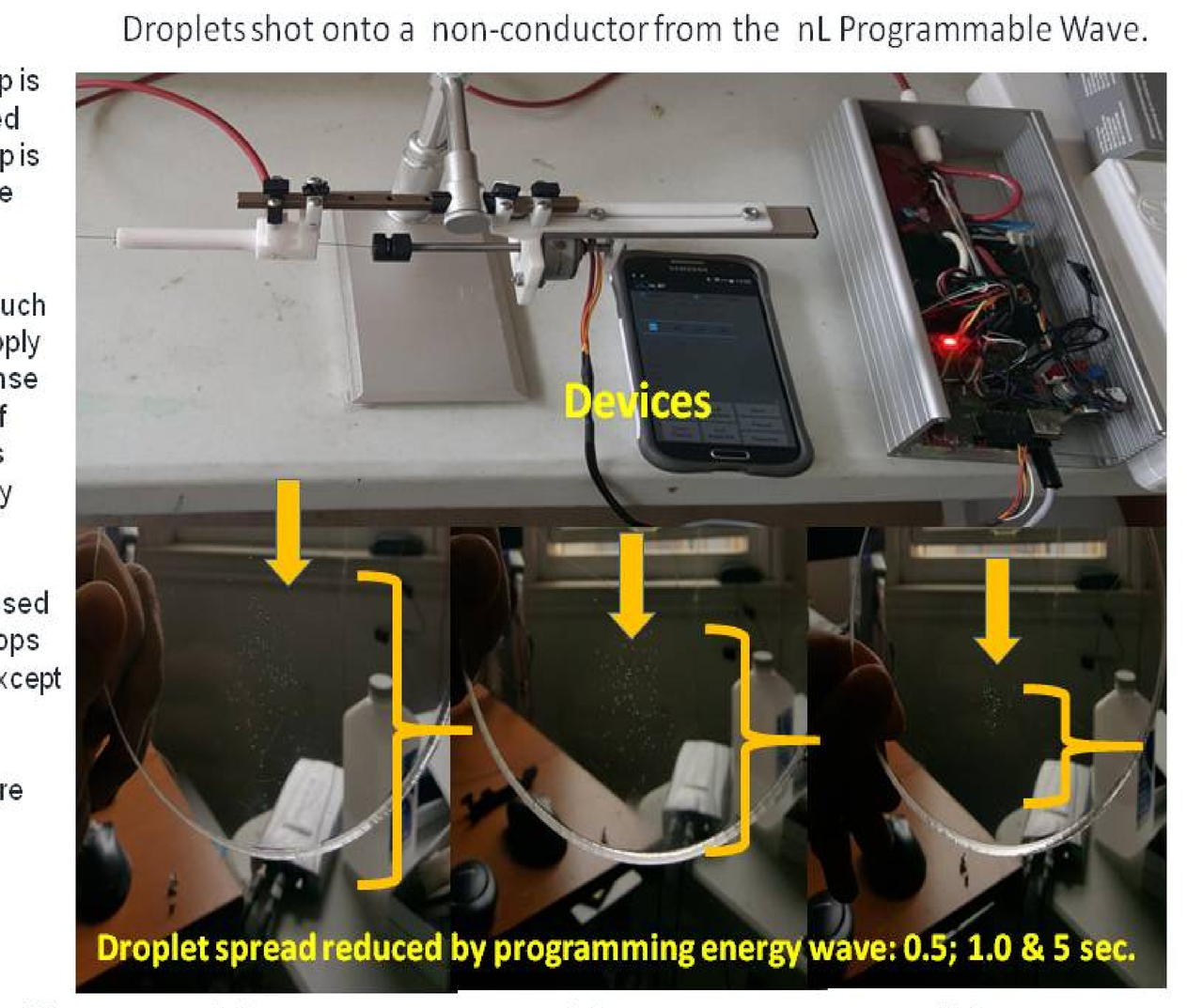
1. Push mode: the drop is same polarity as applied voltage, pull mode: drop is opposite polarity, hence attraction.

2. Push mode, if you touch plunger with skin (or apply any ground) NO dispense will occur. the length of liquid column becomes conductive, presumably due to polarization.

3. Push mode, I have used push mode to shoot drops straight up. no target except for ceiling 8ft away.

4. IBF Energy waves are useful.

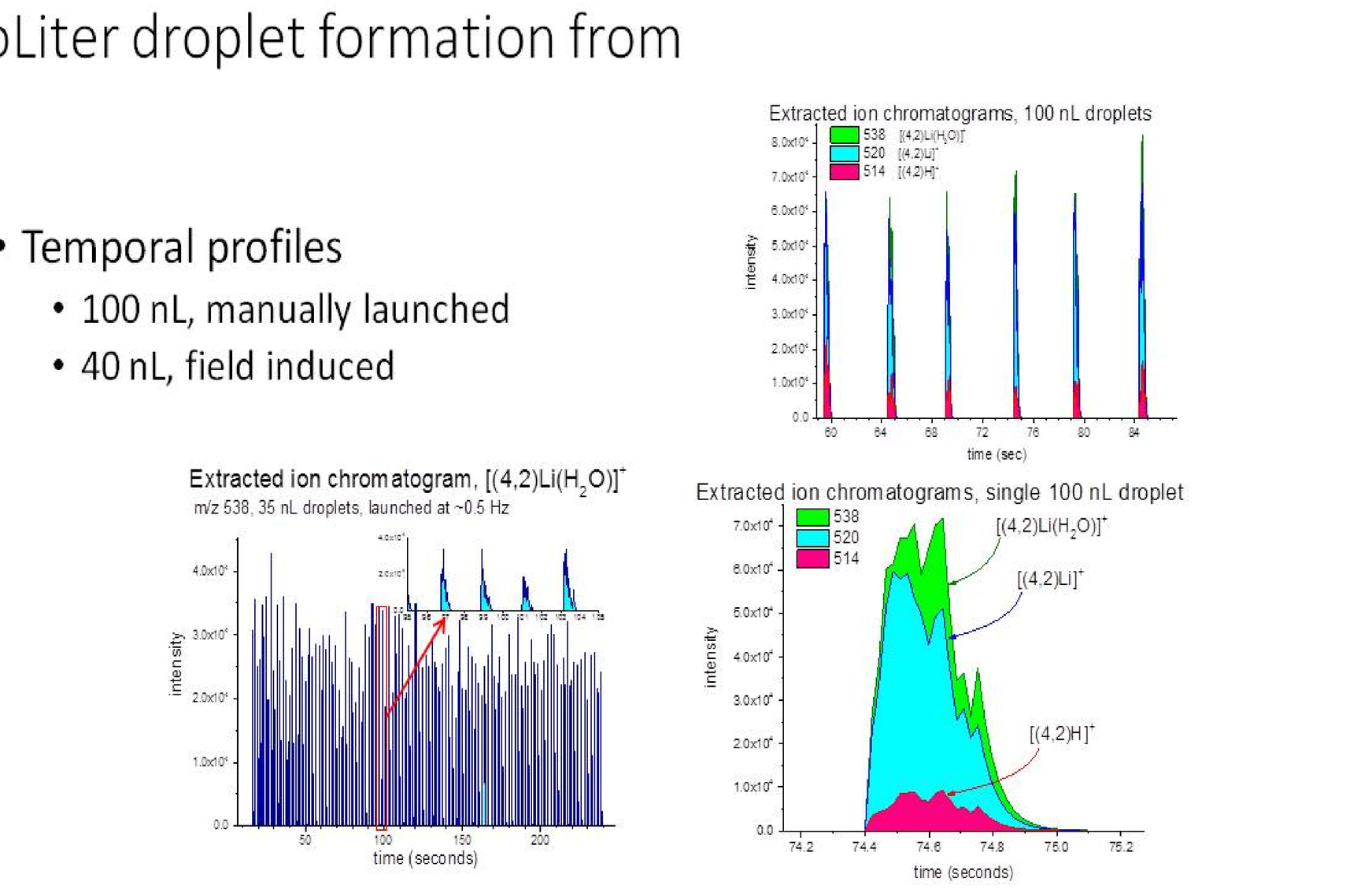
5. Ongoing work just started. Demoed at Stanford, elsewhere.



Droplet dispersion is reduce by 5-10x over the 6 inch travel when energy is programmed. Best wave, timing, etc. tbd.

NanoLiter droplet formation from

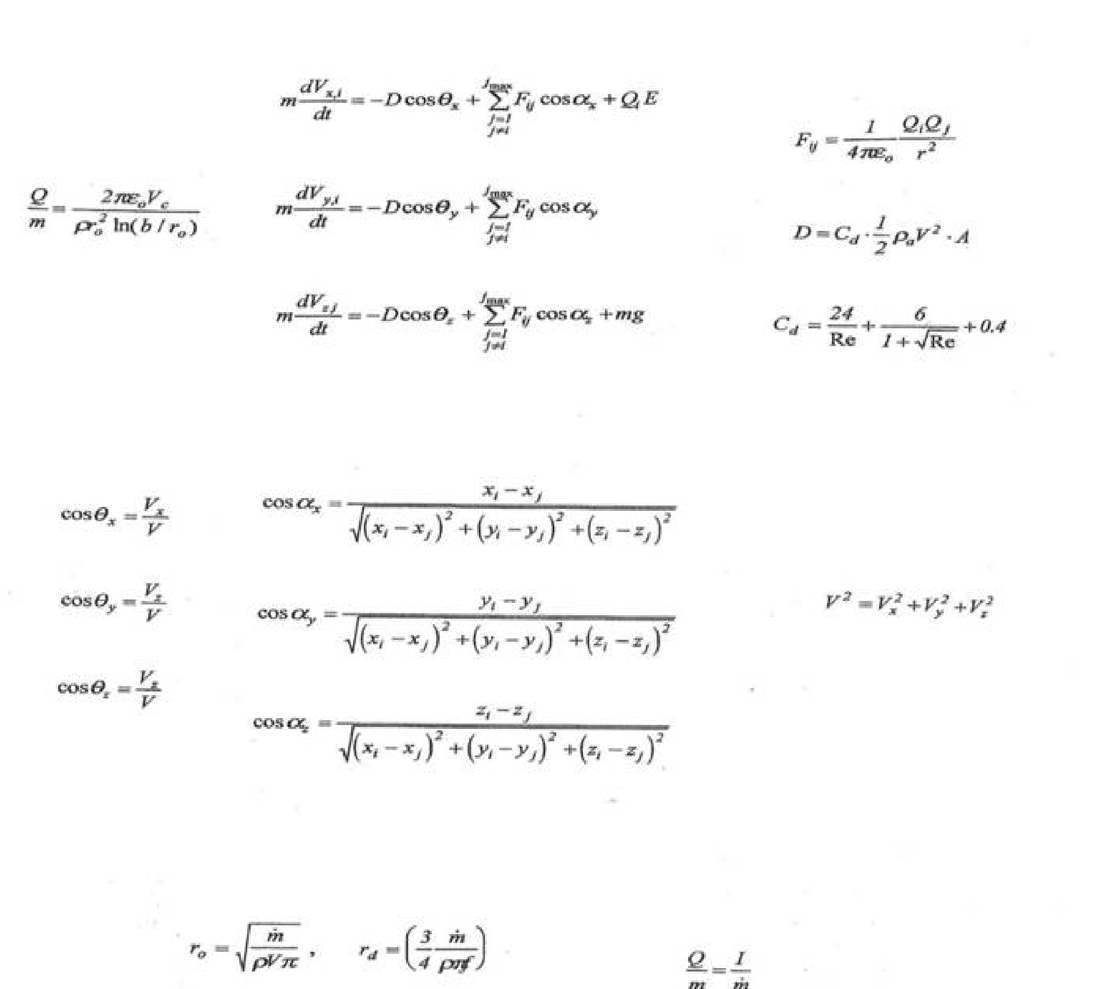
- Temporal profiles
  - 100 nL, manually launched
  - 40 nL, field induced



Equations for Trajectory Calculations of Charged Droplets in the Atmosphere.

See references of A. Scheeline et al, A. Sauter, et al and that of M. Orme, et al.

Note: equations for mass to charge ratio of a charged droplet equations, trajectory equations, coulombic force equations, with geometric and velocity equations and drop geometric relationships.

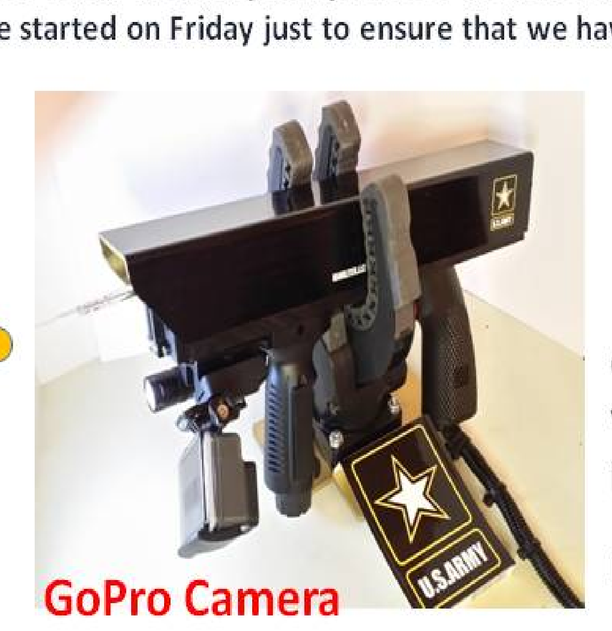


Dispensing nL volumes of agents/simulants **OVER A DISTANCE** for US Army, via the APG-X1, 2016

Drops travel like charged bullets Drops can be shot at ca. 1 m/s up to 1- 2 m via the US Army APG-X1 Dispenser, 3 X 50 nLs, see target below. After "invention" in 2015, the US Army tested the dispensing accuracy of the by Device by using GCMS. See Jon Oyler et al, poster this meeting.

US ARMY. APG,MD. TWO MESSAGES FROM ABERDEEN PROVING GROUND,MD to nanoLiter LLC. August 3, 2015 PARTIAL MESSAGE 1 Classification: UNCLASSIFIED, Caveats: NONE Hi Drew, Got your voice mail today. The nL pipette is working well. We have a procedure to fill it and dispense the volumes we need. We are preparing for a validation run using methyl salicylate. We plan to pipette volumes 5 to 500 nl using 5 replicates. Will quantitate using GCMS. We will keep you posted on our progress.

Classification: UNCLASSIFIED, Caveats: NONE ,Unidentified Project Leader August 24, 2015, PARTIAL MESSAGE 2,Classification: UNCLASSIFIED, Caveats: NONE. We have run curves and controls with very good results over a concentration range from 50 ng/mL to 15 ug/mL. We ran the controls at two concentrations with three replicates each. Accuracy and precision measures were very good (%CVs <5%). We are repeating a stability study today and tomorrow that we started on Friday just to ensure that we have good stability over a five day time period. It looks like we are almost there.
Unidentified Agent Accountable Officer



Can vary frequency, E, volume, wave form & placement.

Distance max = ca. 1.5 m.

Applying Programmed Inductive E Waveforms Improves IBF MS Dispensing Physical Precision.

Best drop accuracy, means one must direct the droplet, but align the droplets initial position.

Note if not aligned, droplets can wobble making the transfer to the MS, less precise than it might otherwise be.

Applied field straightens up the drop, and launches it from same x, y, x position at "same" time done properly. Different functions, rates available! Work ongoing. See below.

Battery fluid conclusions

- Nanodroplet analysis enables glimpses at the intrinsic complex-formation chemistry of the phosphazene cyclotrimers
- Carbonate complexes increase with increasing numbers of $–OCH_2CF_3$ moieties
- Hydrates are preferred for compounds with more $–OC_2H_5$ groups
- Useful approach for systems with very limited sample volume

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BOTTOM LINE

"ANY" Gaussian Surface + ELECTRIC INDUCTION ……
can be an **ION SOURCE** ……. or a ……MALDI, SIMS, LDI or other **DISPENSER,** that's optionally Android controlled.

Finally, as given at ASMS's Asilomar Meeting in 2016 with the University of Cincinnati and Idaho National Laboratory 100% input efficient IBF based UPLC MS of nucleosides yielding similar sensitivities has been observed on the same platform that executes 100% input efficient, millisecond infusion sample input.

Work continues.