Micro-technologies for acquiring biological information

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Commercial Activities

Caliper Life Sciences
- Scientific Founder

revvity

908devices
- Scientific Founder

REPLIGEN

Codetta Bio™
- Scientific Co-Founder
- Director

MOVE ANALYTICAL
- Founding Partner

Ramsey Group Activities

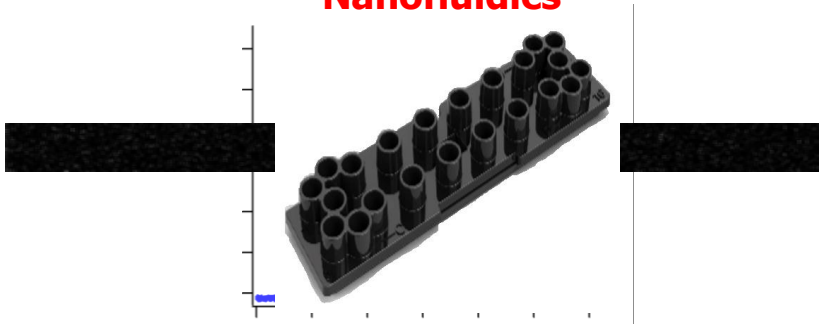
Microfabricated Separations Devices

CE-nESI-MS

µMS Devices

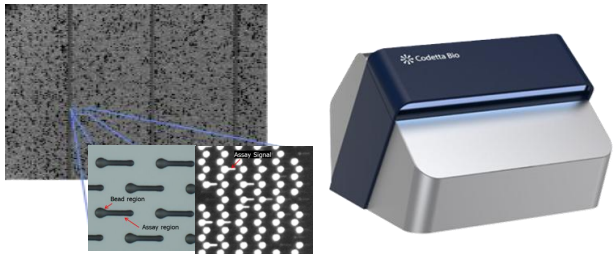
Microfabricated Ion Trap MS Devices

Nanofluidics



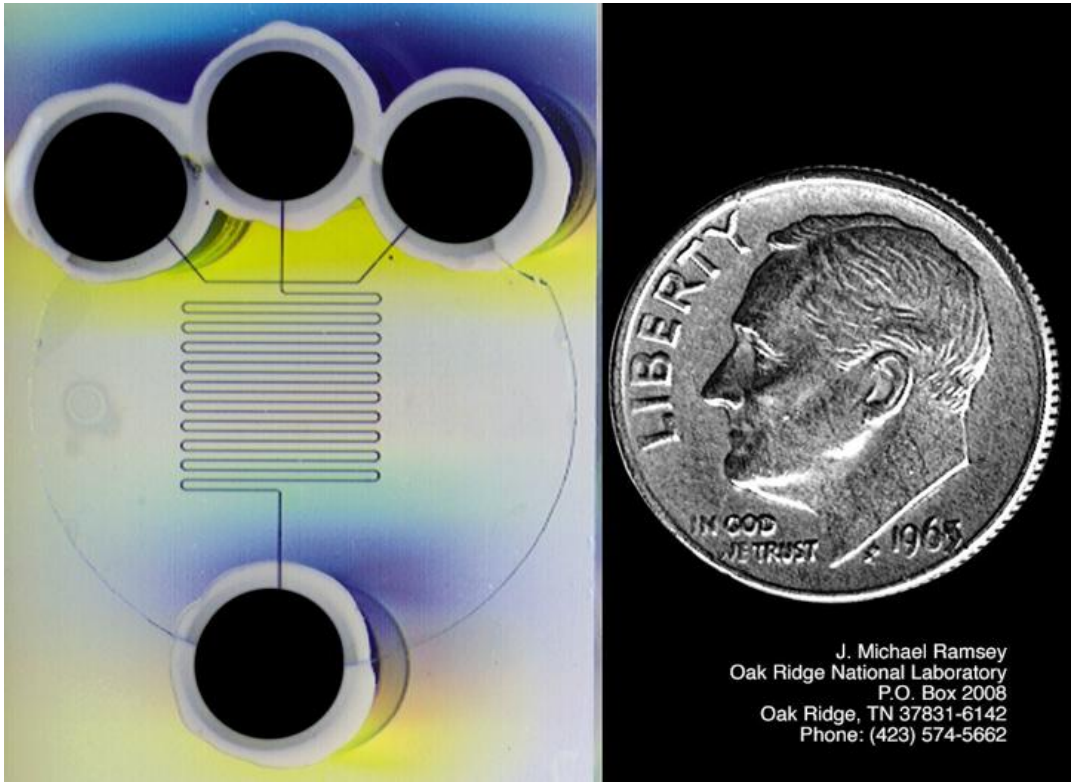
Single Molecule DNA Characterization

Digital Biological Assays



Protein/DNA Multiplexed Assays

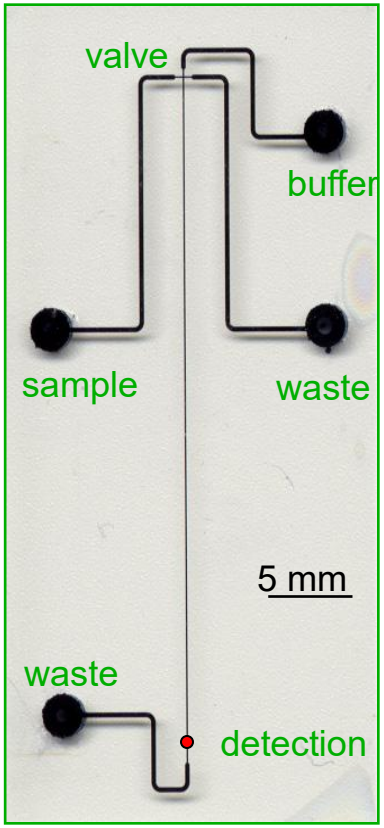
Our First Microchip CE Device

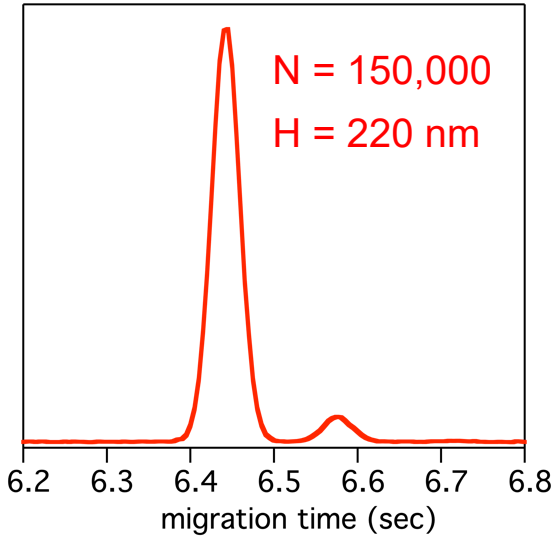


5th HPCE Conference, Orlando, FL, Jan. 25, 1993

Anal. Chem. 66, 1107 (1994).

Rapid Separations in Straight Channel





Analyte: Dichlorofluorescein
Buffer: 20 mM Boric Acid/ 100 mM TRIS
E_{appl} = 1300 V/cm

Caliper-related Microfluidics Products

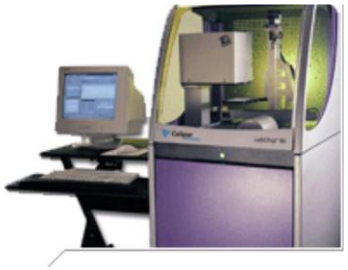
Agilent 2100
Bioanalyzer

2100 Chips

- DNA Sizing
- RNA quantification
- Protein Sizing

Caliper
Kinase
Desktop Profiler

Caliper
LabChip 90

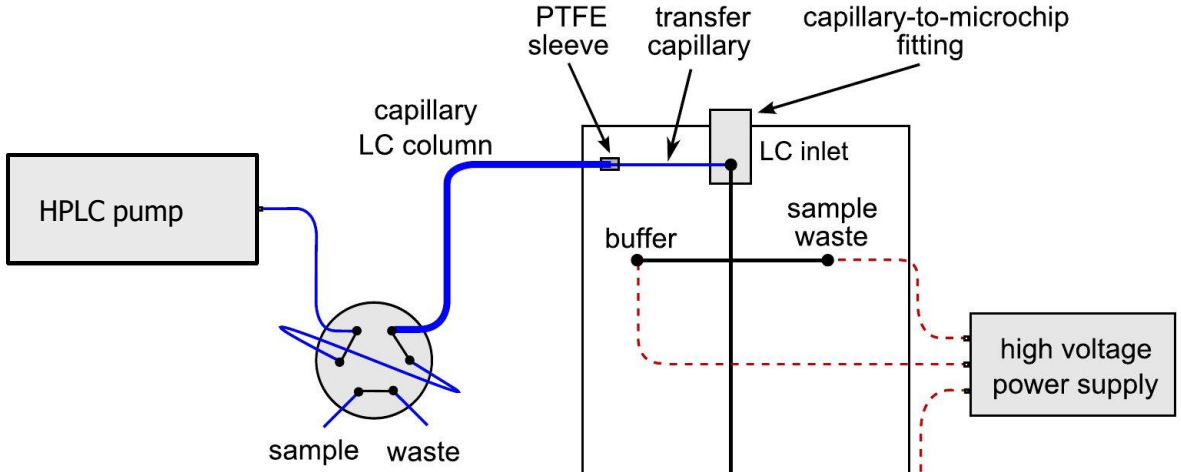


LabChip GXII Touch

Caliper
LabChip 3000

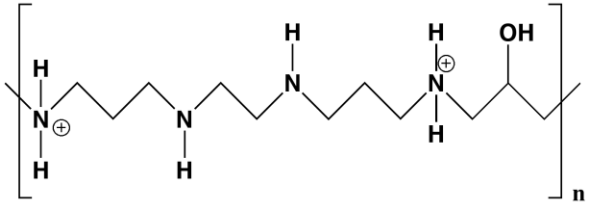
BioRad Experion

2D Hybrid LC-CE-LIF Setup



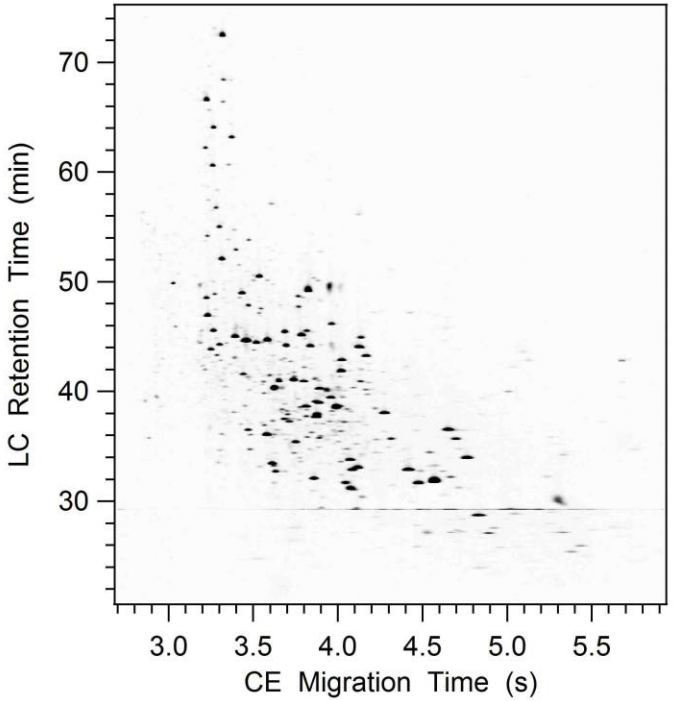
LC column: 75 μm x 54 cm, 2.5 μm C-18

CE channel: 7 μm x 65 μm x 40 mm
coated with PolyE-323*



* Hardenbourg, E. *et al*. *J. Chrom. A* 2003, 1003, 217.

LC-CE-LIF Separation



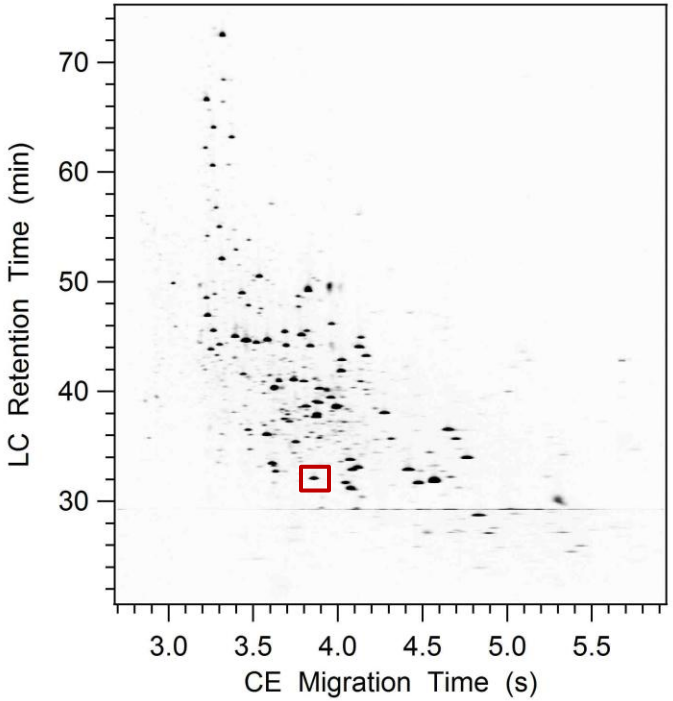
LC: w_b = 21 s, n_c = 134
CE: w_b = 0.043 s, n_c = 58

Total: n_c = 7,772

Effective: n_c ~ 2,500 - 4000

Sample. 1 pmol of bovine serum albumin tryptic digest labeled with TRITC
Liquid chromatography. 75 μm i.d., L=54 cm, 2.5 μm C18 particles. Flow rate: 150 nL/min.
Gradient: 5-50% B in 60 min. (A, 0.1% formic acid; B, 0.1% formic acid in acetonitrile)
Capillary electrophoresis. 1% formic acid, 25% acetonitrile. L= 2.3 cm, E= 1.5 kV/cm

LC-CE-LIF Separation



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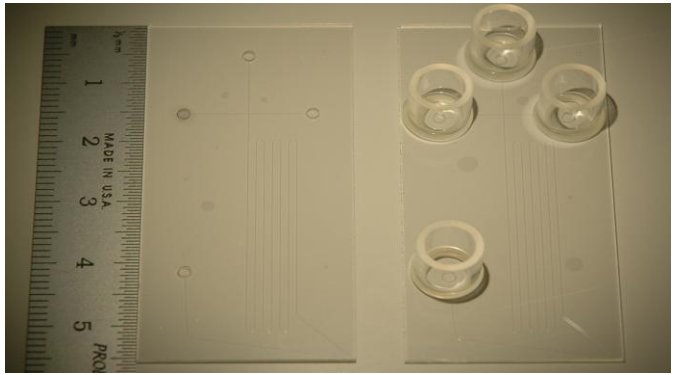
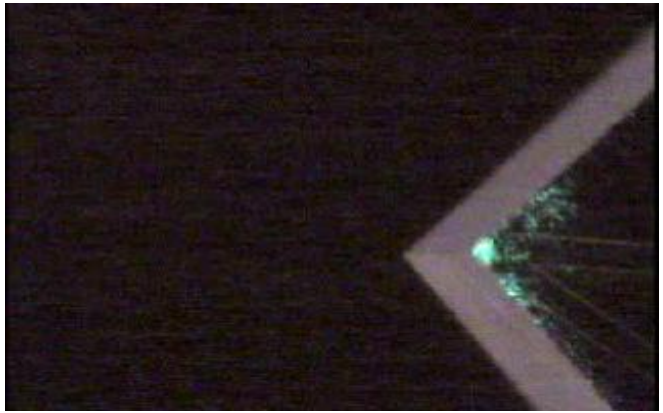
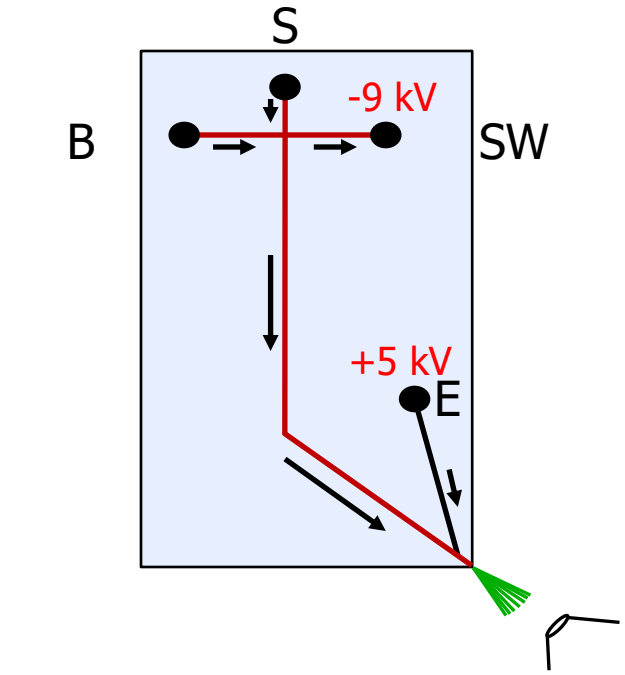
Microchip CE-ESI Device

Monolithically Integrated nanoESI Emitter





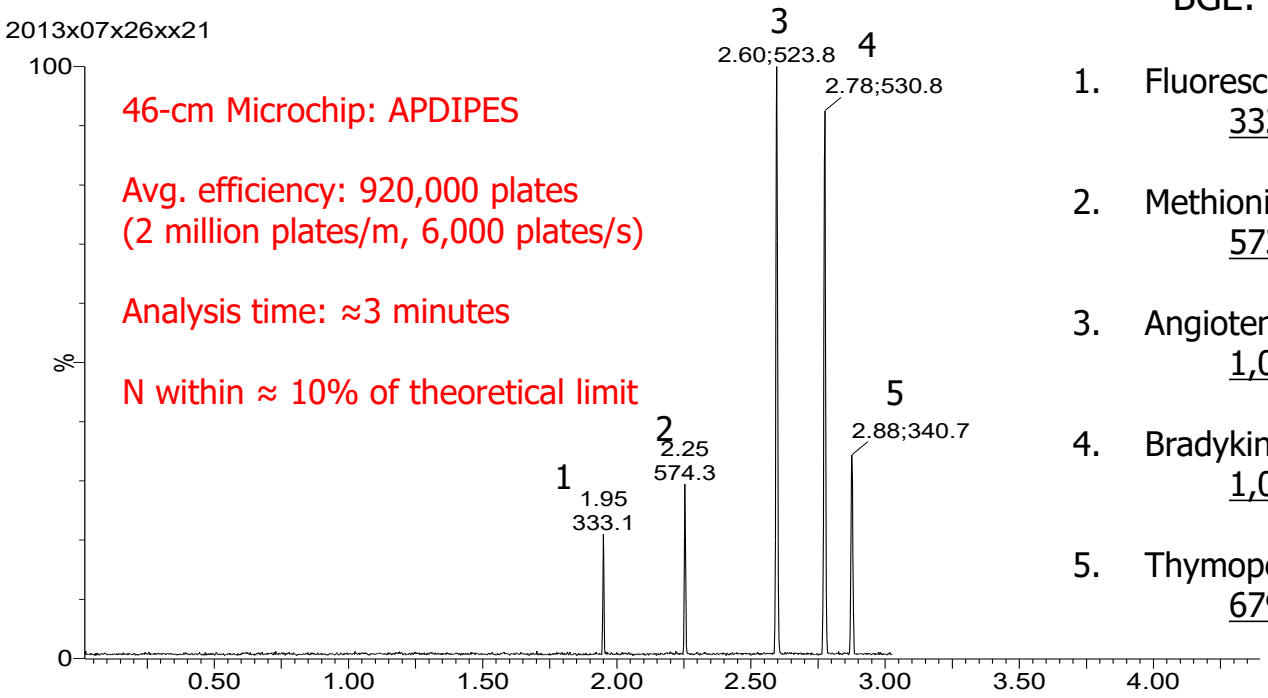


Positive surface charge

Negative surface charge

R.S. Ramsey, et al, Anal. Chem., 69, 1174 (1997), J.S. Mellors et al., Anal. Chem. 2008. *80,* p. 6881-6887

CE-MS of Peptides: CVD-APS Surface



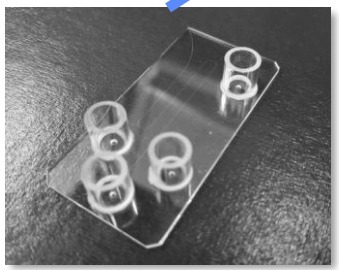
Peptide Standard Mix 5 μM
BGE: 50% ACN, 0.1% FA
400 V/cm

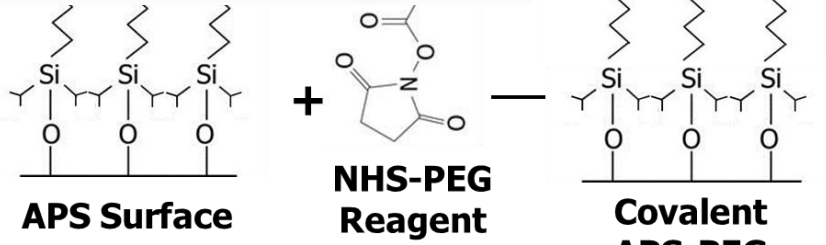
1. Fluorescein
   332.3 Da
2. Methionine Enkephalin
   573.8 Da
3. Angiotensin II
   1,046.2 Da
4. Bradykinin
   1,060.2 Da
5. Thymopentin
   679.8 Da

0.5 nL injection 170 nL/minute

N.G. Batz, J.S. Mellors et al., *Anal. Chem.* 2014, *86,* 3493-3500.

Microchannel Surface Chemistry is Crucial





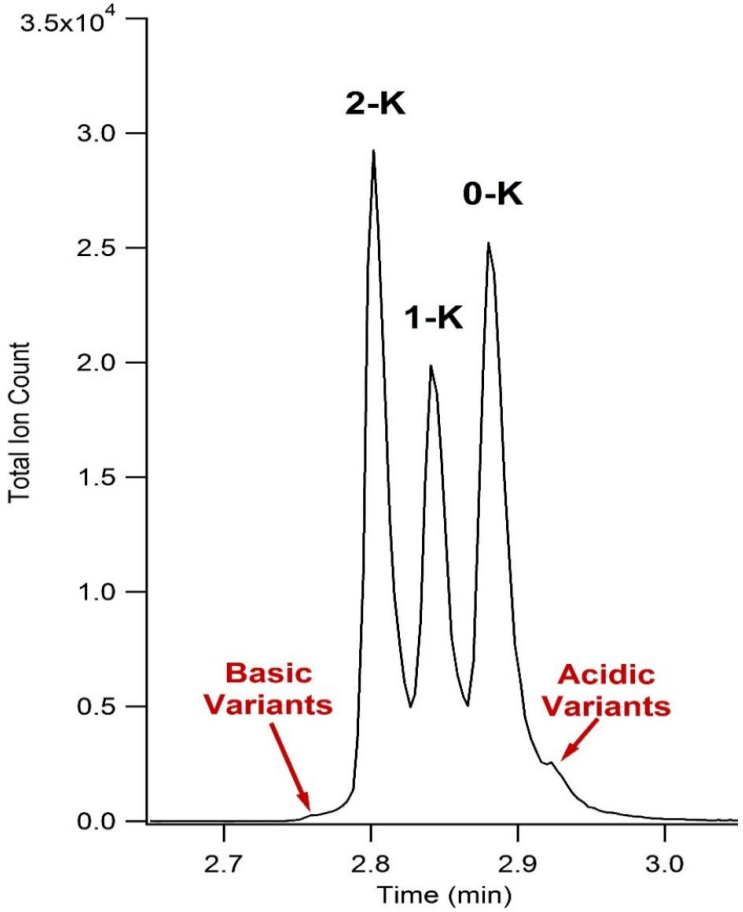
Requirements:

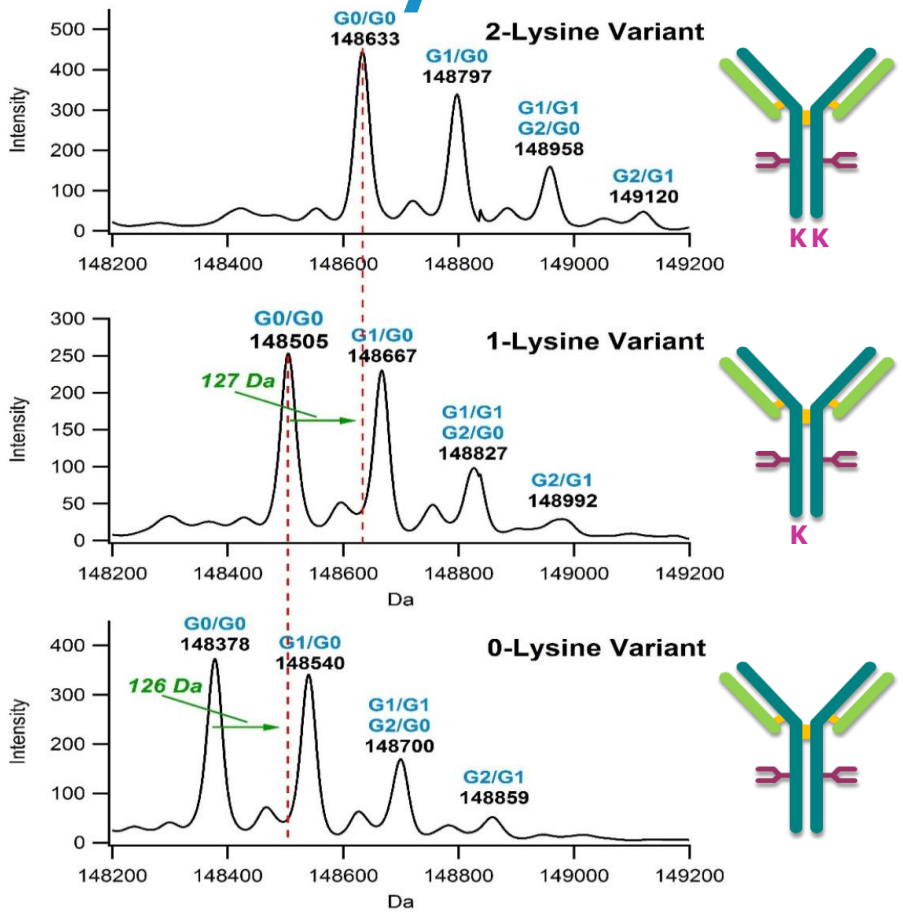
- Suppress electroosmotic flow (EOF)
- High uniformity
- Prevent analyte adsorption

N.G. Batz, J.S. Mellors et al., *Anal. Chem.* 2014, *86,* 3493-3500.

E.A. Redman, J.S. Mellors et al., *Anal. Chem.* 2015, *87,* 2264-2272.

Intact Infliximab Analysis

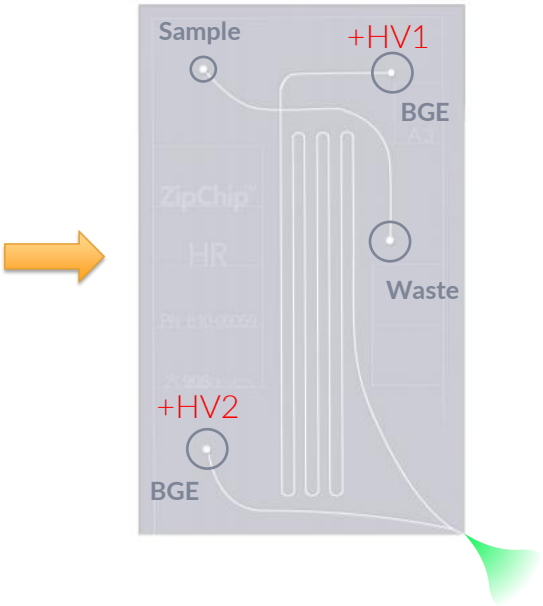




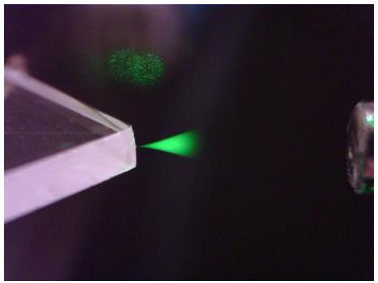
Redman, E. A.; Batz, N. G.; Mellors, J. S.; Ramsey, J. M. *Anal. Chem.* 2015, *87* (4), 2264–2272.

The ZipChip Platform

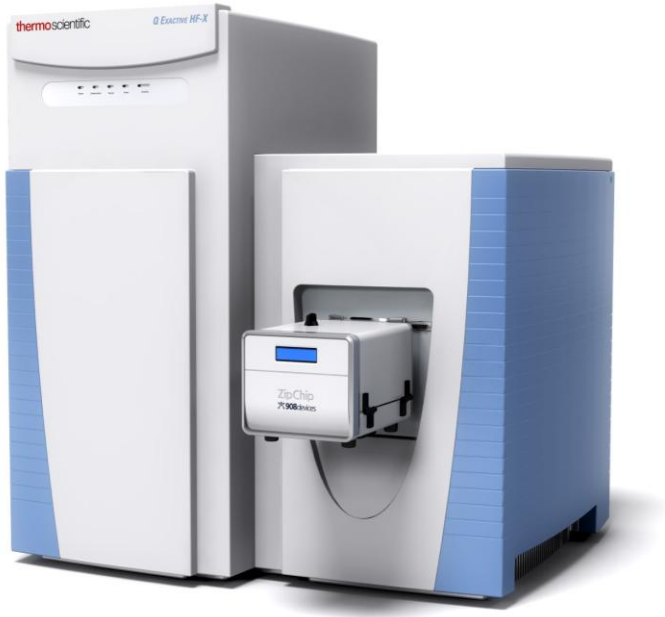
Glass microfluidic chip
in housing



- Integrated microfluidic platform
- Zone electrophoresis separations
- Integrated ESI emitter
- ESI flowrate ~150 nL/min
- Highly uniform and stable surface coatings for high resolution separations



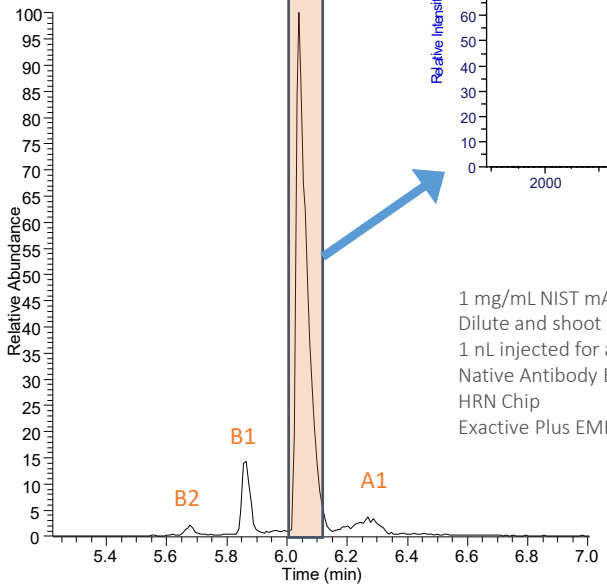
What is ZipChip?



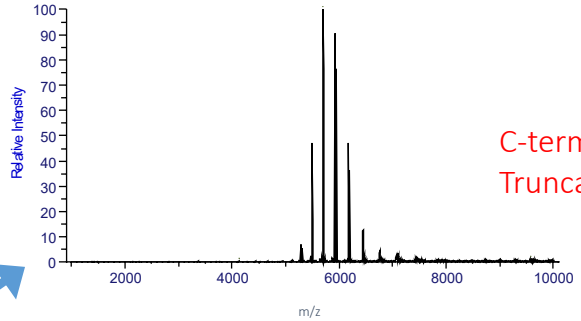
Bruker and Sciex also

908devices

ZipChip Native Antibody Analysis



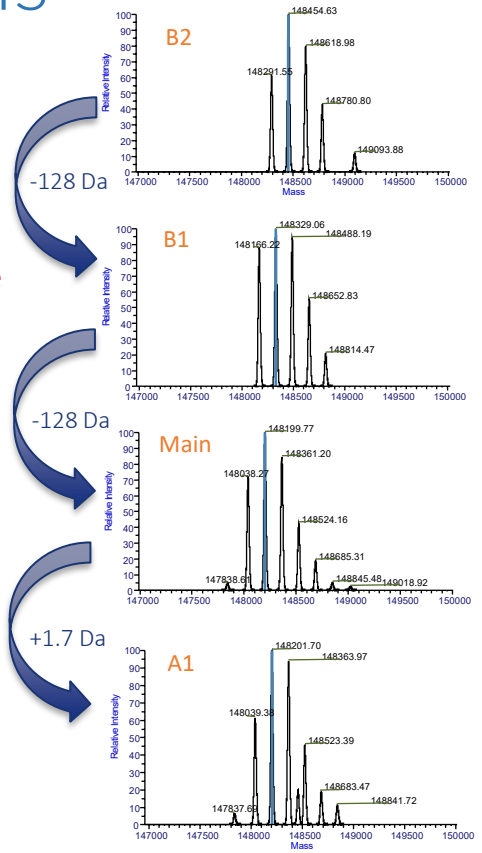
Fully native spectra for all charge variants



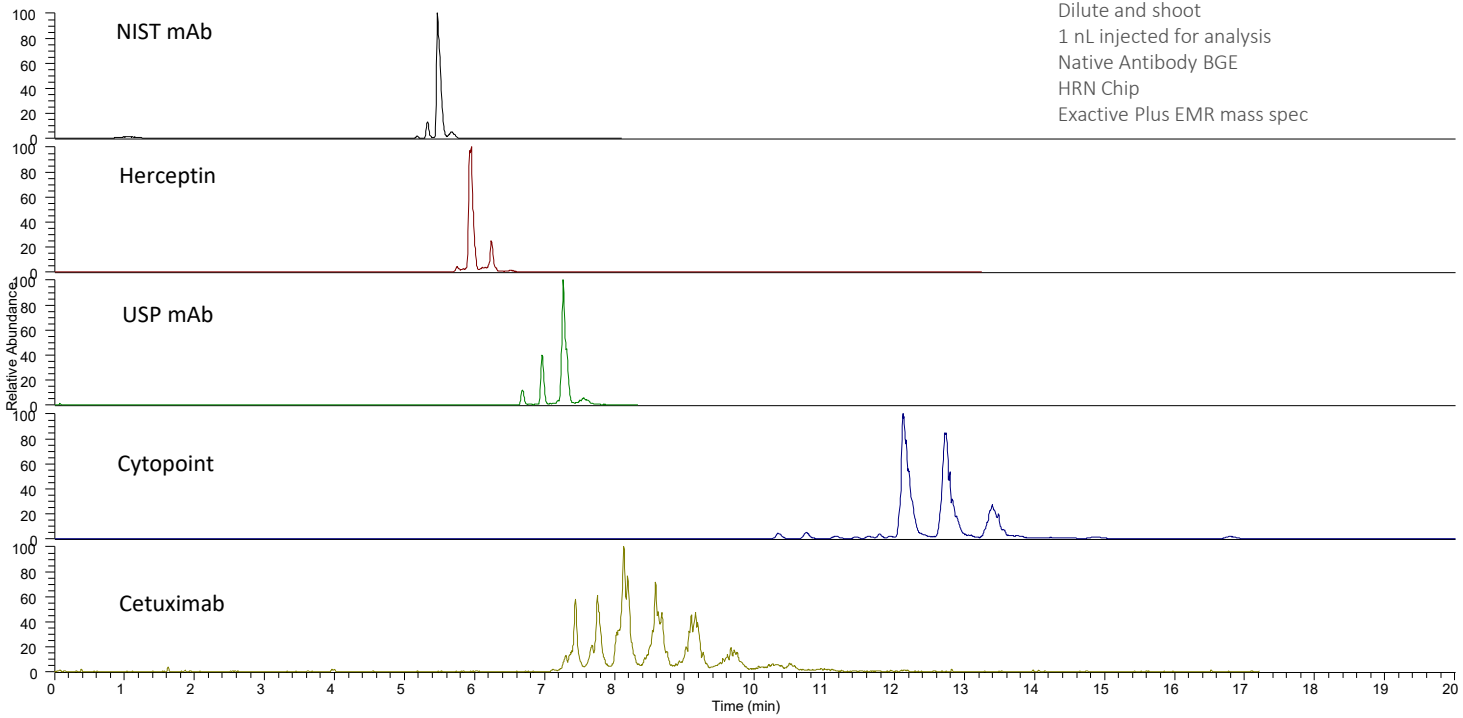
1 mg/mL NIST mAb
Dilute and shoot
1 nL injected for analysis
Native Antibody BGE
HRN Chip
Exactive Plus EMR mass spec

C-terminal Lysine Truncation

deamidation

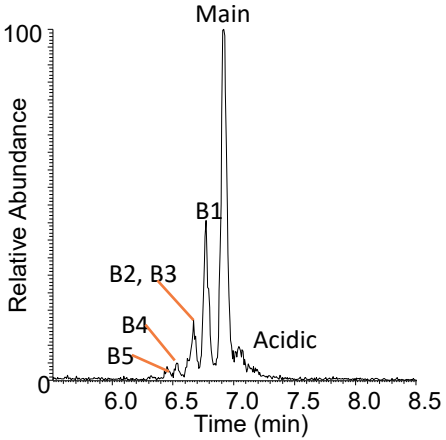


Universal Method for Native mAb and ADC Analysis

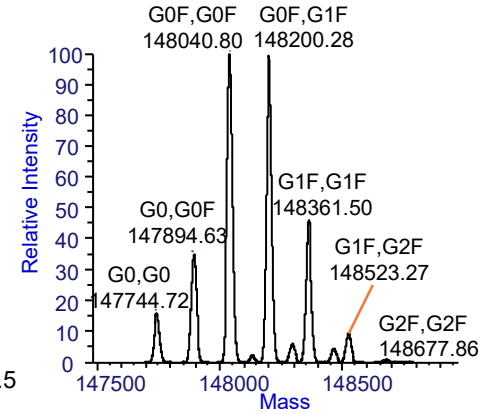


PQA Analysis from a Fed-batch Bioreactor

- Spent media samples were collected twice daily from a bioreactor culture of NISTCHO expressing NISTmAb
- 50x dilution of spent media prior to ZipChip CVA
- Data processing in BioPharma Finder and UniDec
  - > Mass shifts between the charge variants and shifts in migration times are used to identify PTMs
  - > Mass shifts within the deconvoluted spectra of the main variant were used to identify glycoforms



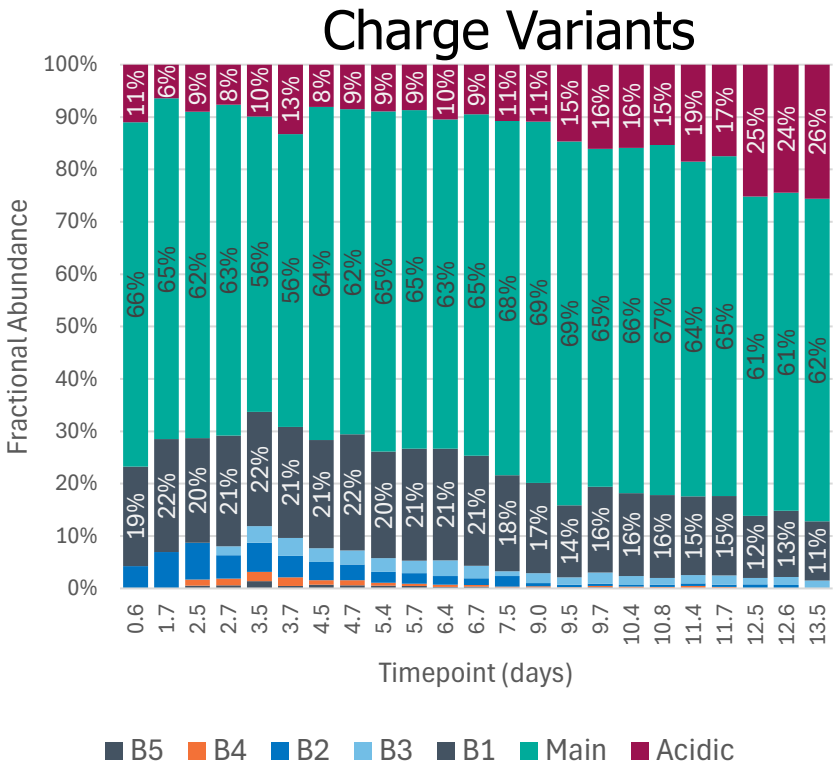
Charge variant profile from ZipChip separation.

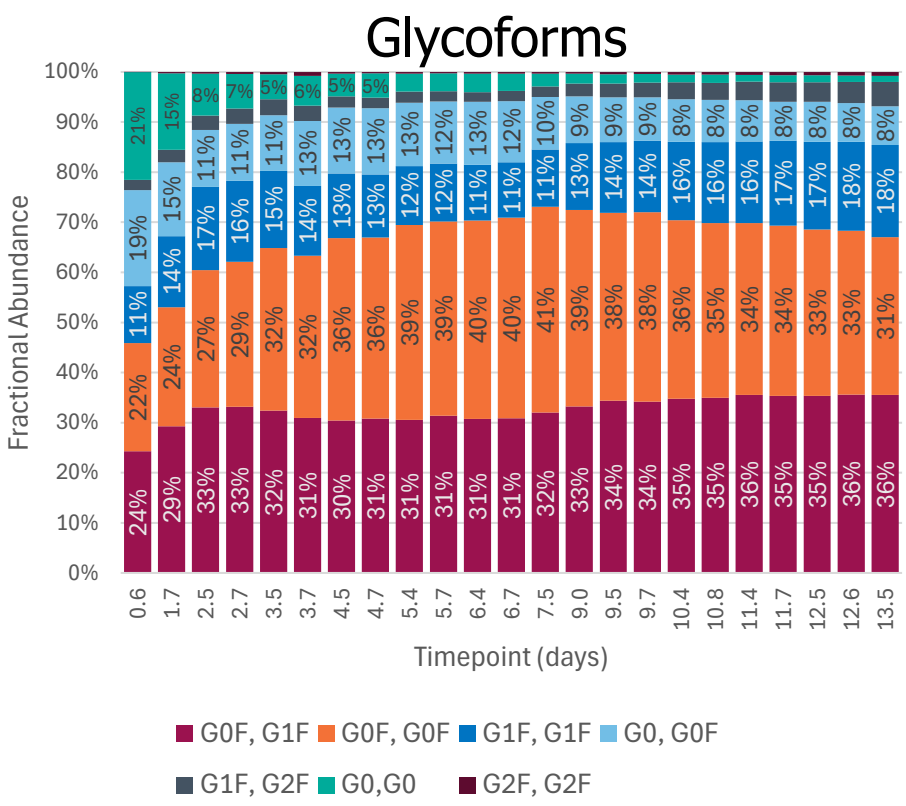


Processed MS of Main variant including masses and glycoform IDs.

Variant	PTM Identification	
B5	2x pyroE, 2x C-am	pyroglutamic acid, C-terminal amidation
B4	1x pyroE, 1x C-am	pyroglutamic acid, C-terminal amidation
B3	0x pyroE	pyroglutamic acid
B2	2x pyroE, 1x C-am	pyroglutamic acid, C-terminal amidation
B1	1x pyroE	pyroglutamic acid
Main	2x pyroE	pyroglutamic acid
Acidic	deamidation	

Monitor PQAs Throughout Cell Culture





Automated Sampling Toward Closer Integration

Trace filtration probe and pump

Spark Holland Integrity Autosampler

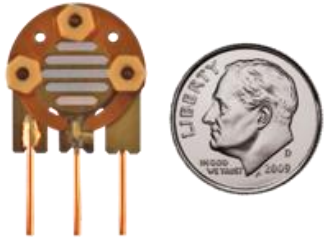
Media stream sampled every hour

Courtesy of Katherine Raiche

Miniaturizing CE-MS

Centralized Laboratory

Versatility
Random Access



HPMS

Benchtop
Manufacturing Floor

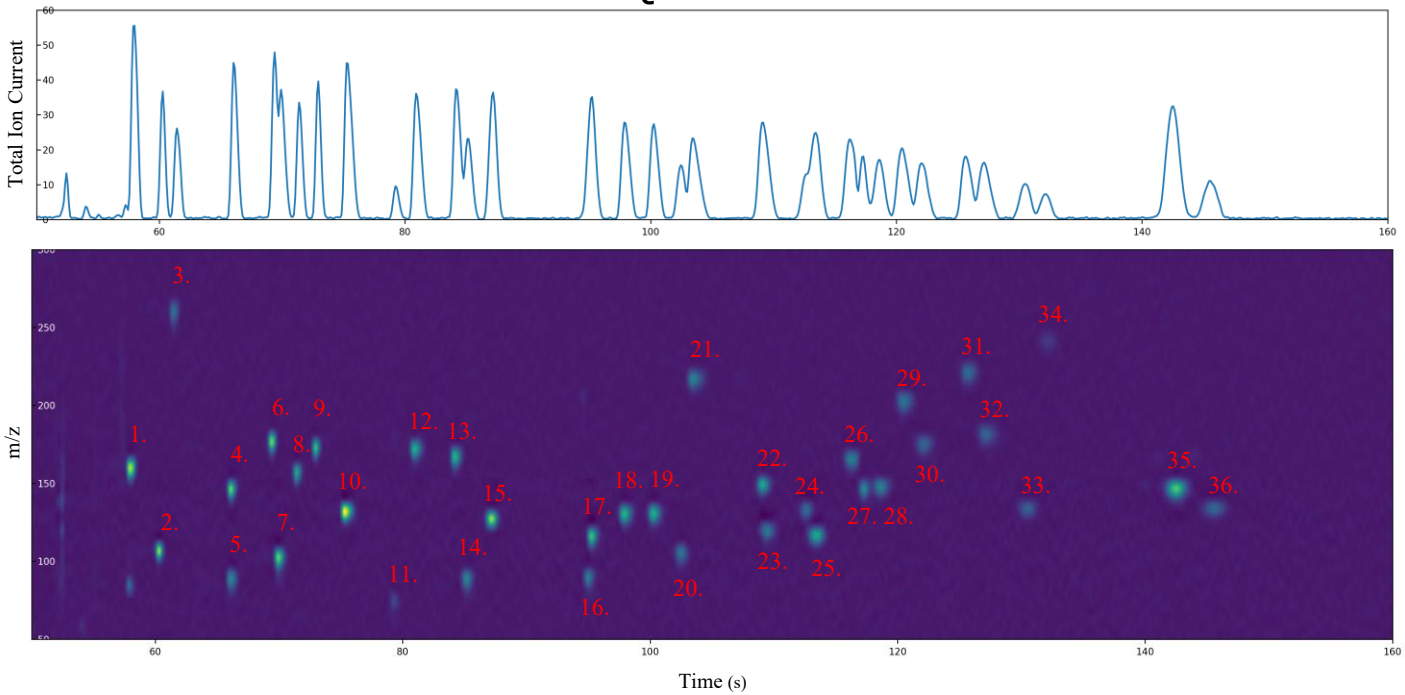
Ease of Use
Answer Driven

Microfluidic CE-ESI

W. M. Gilliland, J. S. Mellors, and J. M. Ramsey, Anal. Chem., 2017, 89, 13320-13325

CE-MS Image Plot

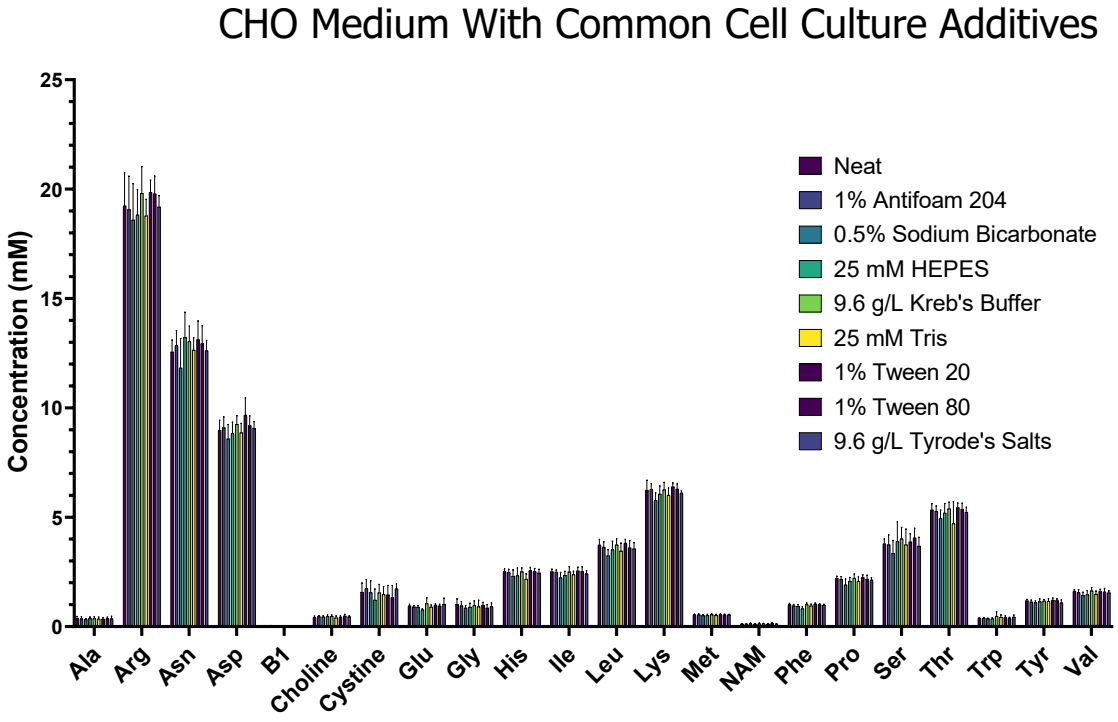
Performance Qualification Standard



1-Internal Standard, 2-Choline, 3-Vitamin B1, 4-B-Alanine, 5-Lysine, 6-Arginine, 7-GABA, 8-Histidine, 9-Methylhistidine, 10-Internal Standard, 11-Glycine, 12-Vitamin B6-OH, 13-Vitamin B6-Oxo, 14-Alanine, 15-Nicotinamide, 16-Sarcosine, 17-Valine, 18-Isoleucine, 19-Leucine, 20-Serine, 21-Alanyl-Glutamine, 22-Methionine, 23-Threonine, 24-Asparagine, 25-Proline, 26-Phenylalanine, 27-Glutamine, 28-Glutamic Acid, 29-Tryptophan, 30-Citrulline, 31-Internal Standard, 32-Tyrosine, 33-Aspartic Acid, 34-Cystine, 35-Internal Standard, 36-Hydroxyproline

Quantitation Tolerant to Common Additives

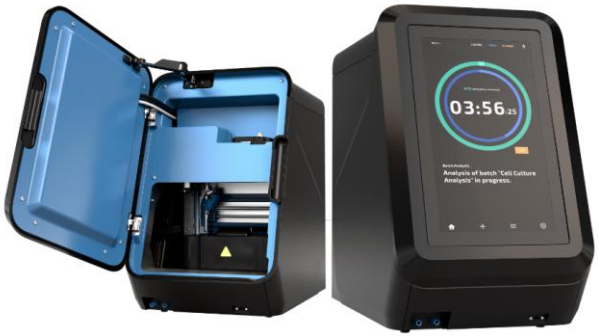
- CHO media spiked with common cell culture additives at specified levels
- Samples were diluted 100x and analyzed with n=5 replicates
- Variability between neat and spiked samples was under 6 %, within experimental error
- Demonstrates reliability despite the simple dilute and shoot sample prep



REBEL XT At-line Media Analyzer

A dedicated cell culture media analyzer designed for rapid and efficient amino acid analysis.

REBEL detects and quantifies a panel of amino acids, dipeptides, and biogenic amines, at-line, with minimal sample prep.



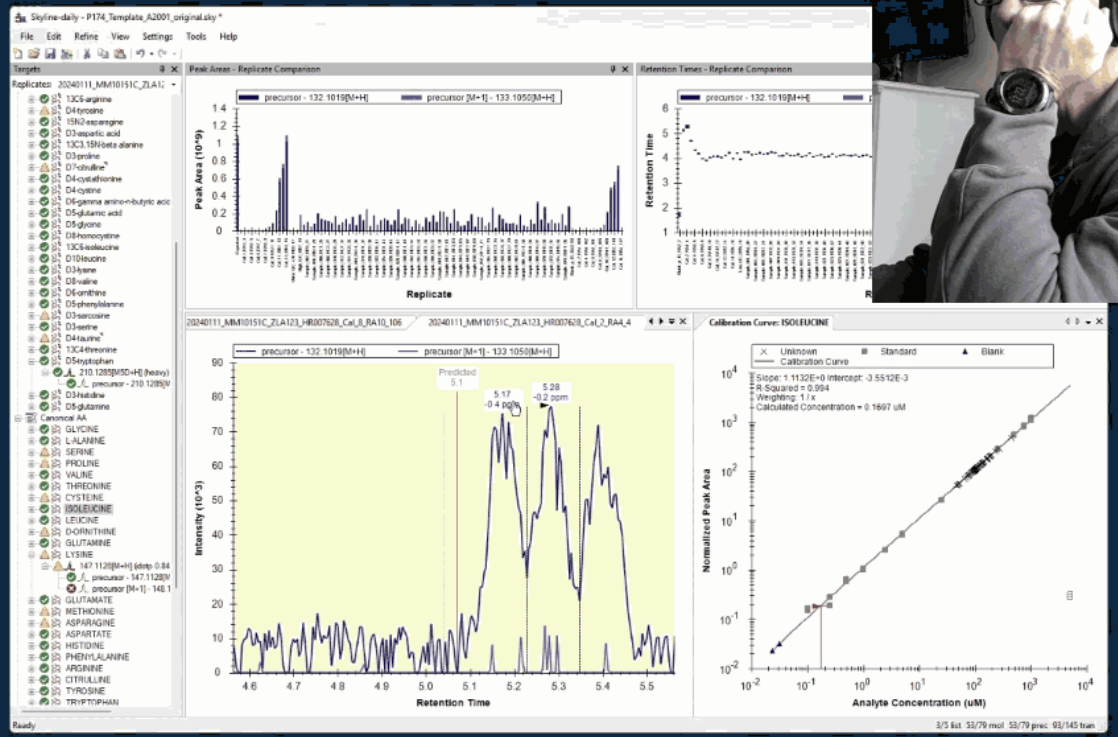
REBEL XT ANALYTE PANEL

- Alanine
- Alanyl-Glutamine
- Arginine
- Asparagine
- Aspartic acid
- Choline
- Cystine
- Glutamic acid
- Glutamine
- Glycine
- Histidine
- Isoleucine
- Leucine
- Lysine
- Methionine
- Phenylalanine
- Proline
- Serine
- Threonine
- Tryptophan
- Tyrosine
- Valine

REPLIGEN

Metabolomics Made Easy.

The Problem



Steep Training Curve

Lengthy Method Dev

Difficult sample prep

Manual peak assignment

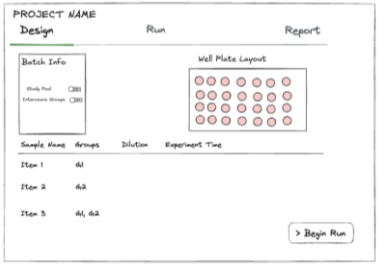
Tedious Reporting

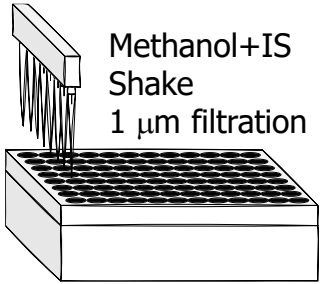
While extremely valuable, metabolomics is:
Difficult to implement and *tedious to perform*

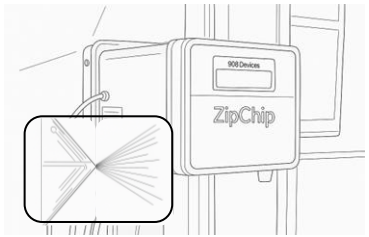
Our Approach

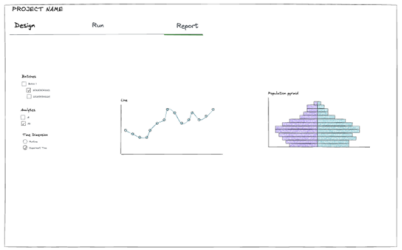
Drive Simplification Wherever Possible.

Design → Prep → Run → Report









5 mins

Intuitive UI / Design
Assign Key Parameters
Export

30 mins/plate

No derivatization
Manual or automated
Everything included

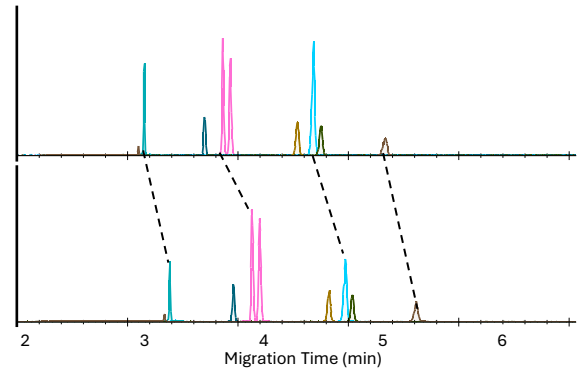
12 mins/sample

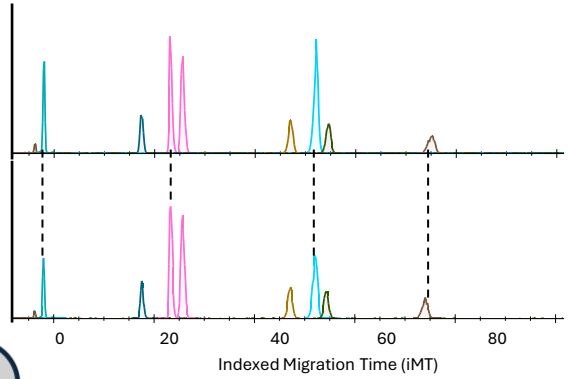
Push-button
Microchip CE-MS
Low matrix effects
High sensitivity

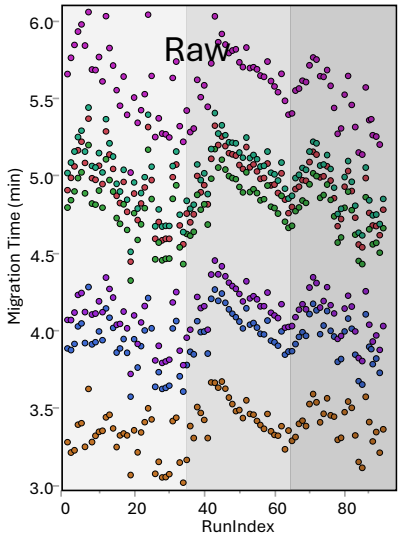
10 mins/plate

Go/No Go SST
Automated Data Check
Quantitative Output

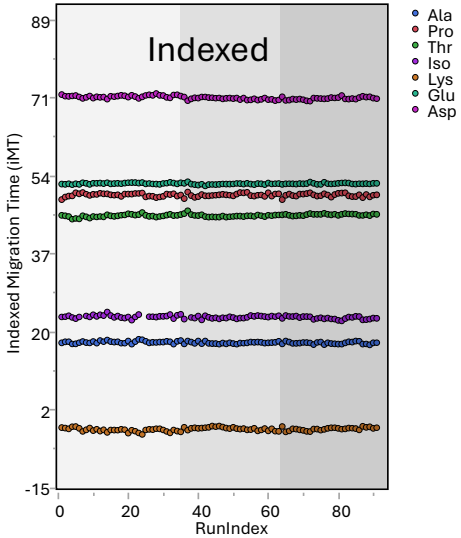
Migration Time Indexing







≈4% RSD

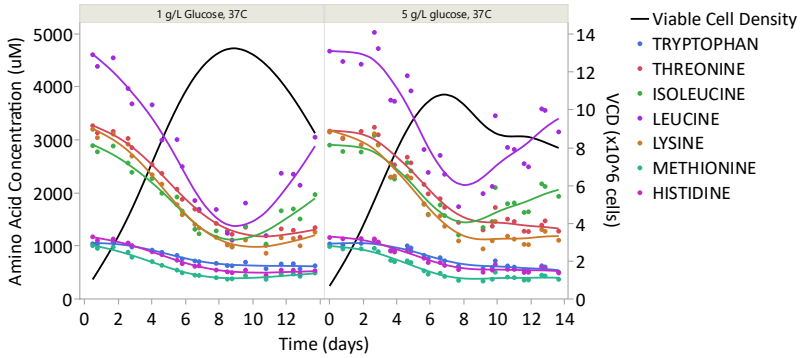


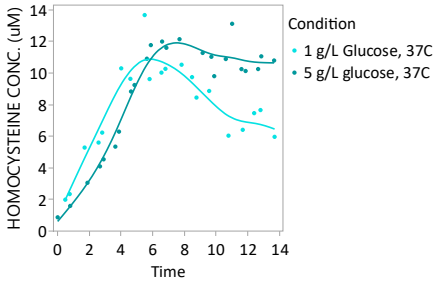
<0.5% RSD

For ZipChip separations, raw migration times vary, but the underlying separation is highly reproducible because it depends only on charge and size of molecules in a static background electrolyte. The raw variance is eliminated by indexing migration times to the migration times of a set of easy to identify internal standards

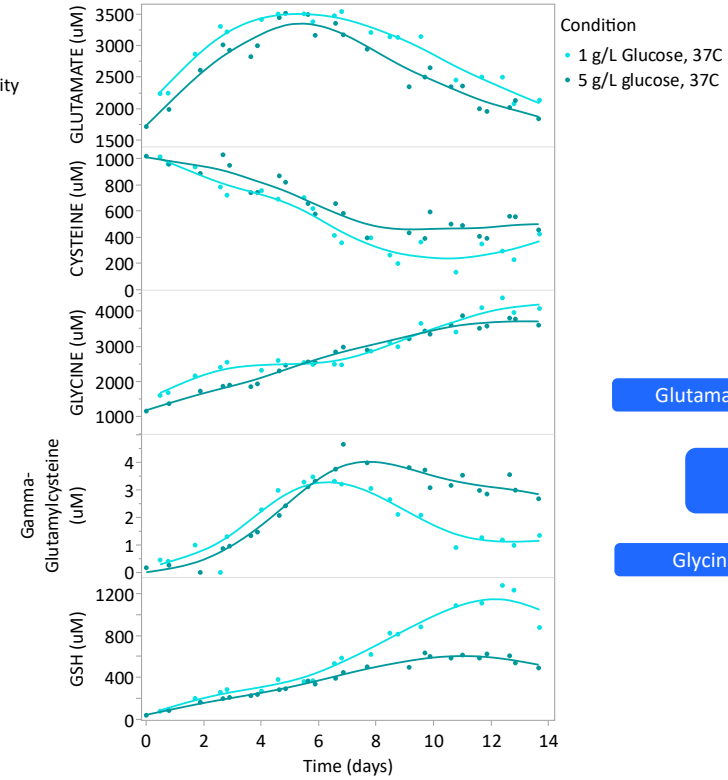
Unlocking Data Exploration – Bioreactor Broth Analysis

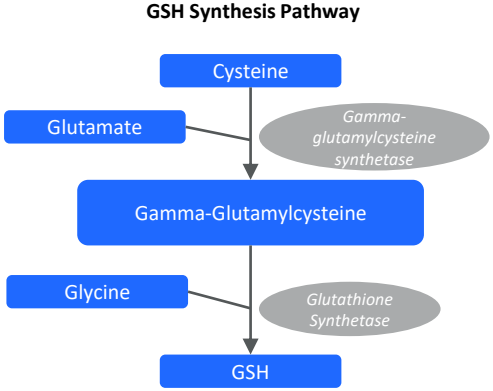
NISTCHO expressing NISTmAb
Continuous fed reactor





Homocysteine can inhibit cell growth

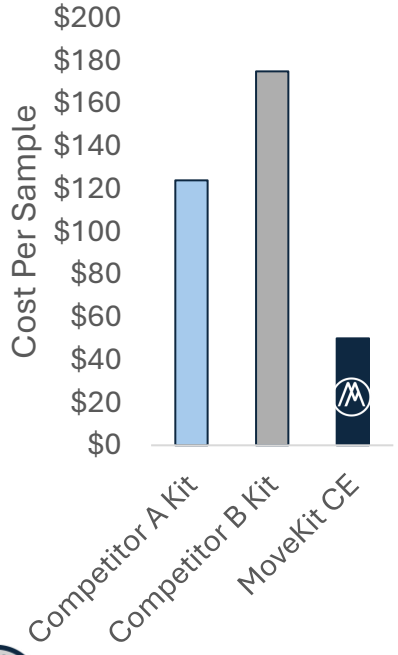




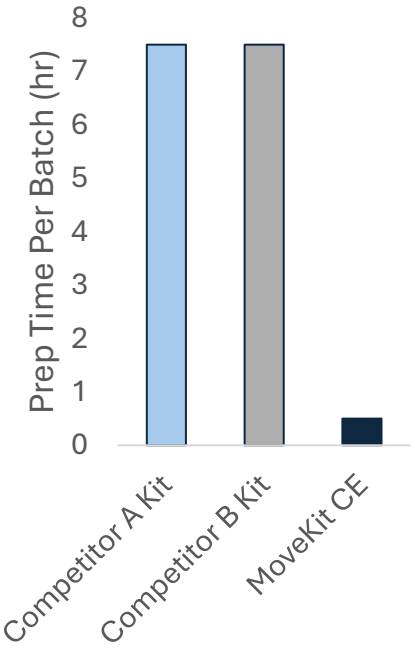
Upregulated GSH linked to higher mAb production.

MoveKit™ Advantages

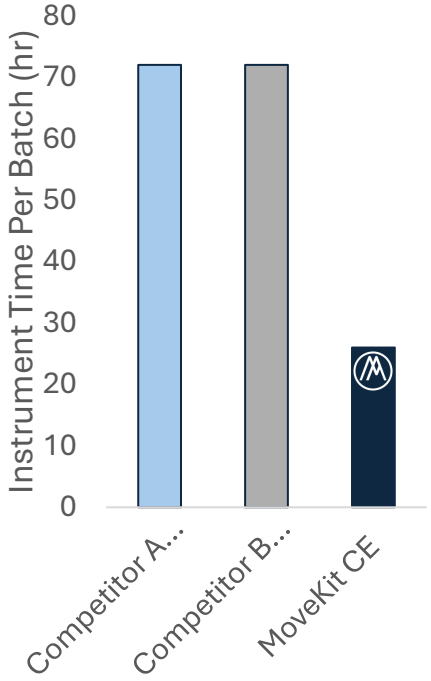
Kit Cost Per Sample



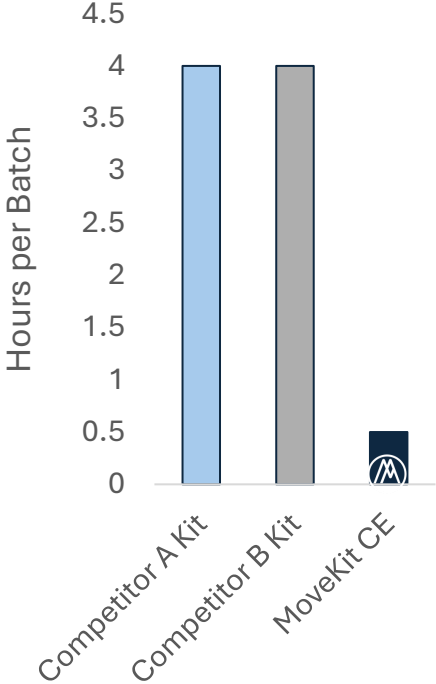
Prep Time per Batch



Inst Time per Batch



Data Processing Time



MoveKit™ Details

What's Included:
Extraction and collection 96-well plates
Extraction and Focusing Reagent
Internal standards (36 SIL)
SST, Blank, Calibration, and QCs

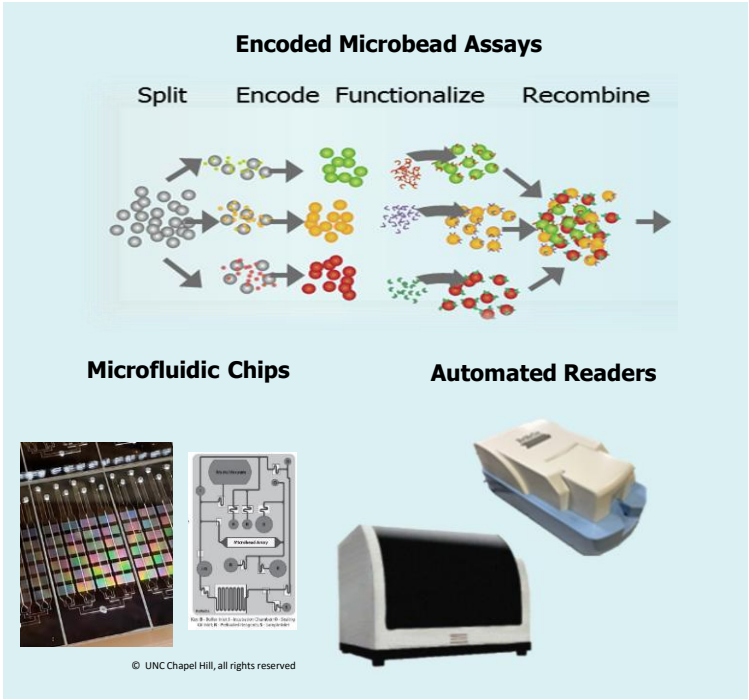
Protocol
20 µL sample (10:1 diluted for media)
180 uL of extraction+focusing reagent
Shake (15 min)
Filter (spin or vacuum manifold)

Coverage:
Library of 300+ polar metabolites including 14 metabolite classes; no derivatization, so flexibility to add more (drugs, exposure…)

Software
Automated experimental design, analysis, reporting, and visualization

A Multi-omic Microfluidic Platform for Bioassays

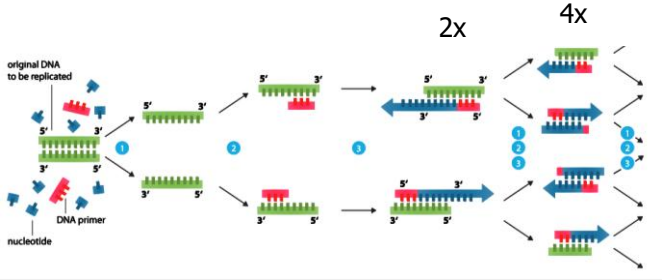
- A differentiating duel digital/analog molecular and protein assay strategy
- Large dynamic range (≈10^8)
- Massively parallel singleplex assays simplify content development process
- High levels of multiplexing in space
- Platform configurations span high-throughput to sample-to-answer
- Small sample volumes addressable (<100 µL)
- State-of-the-art LODs



Nucleic Acid and Protein Assays

The Bioanalysis Workhorses

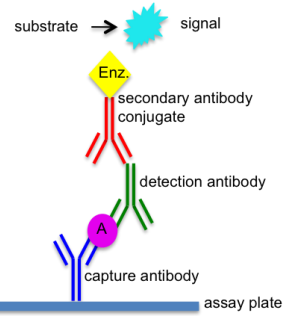
PCR



- Enzymatically mediated geometric amplification
- Specificity provided by NA hybridization
- Enables high sensitivity and specificity assays of NA targets

https://commons.wikimedia.org/wiki/File:Polymerase_chain_reaction.svg#/media/File:Polymerase_chain_reaction.svg

ELISA



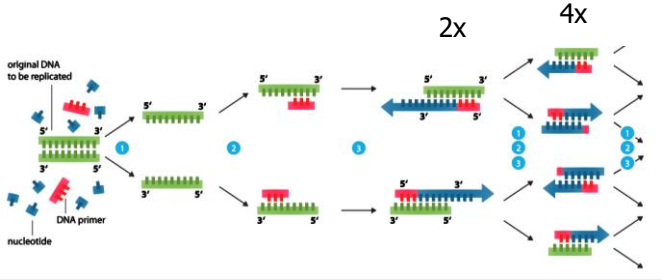
- Enzymatically mediated linear amplification
- Specificity provided by Ab-Ag recognition
- Enables high sensitivity and specificity assays of protein targets

https://www.nordicbiosite.com/blog/elisa-principles-101

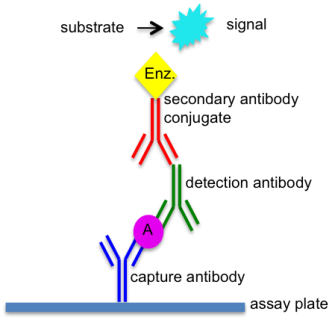
Nucleic Acid and Protein Assays

The Bioanalysis Workhorses

PCR



ELISA



Notable Performance Issues

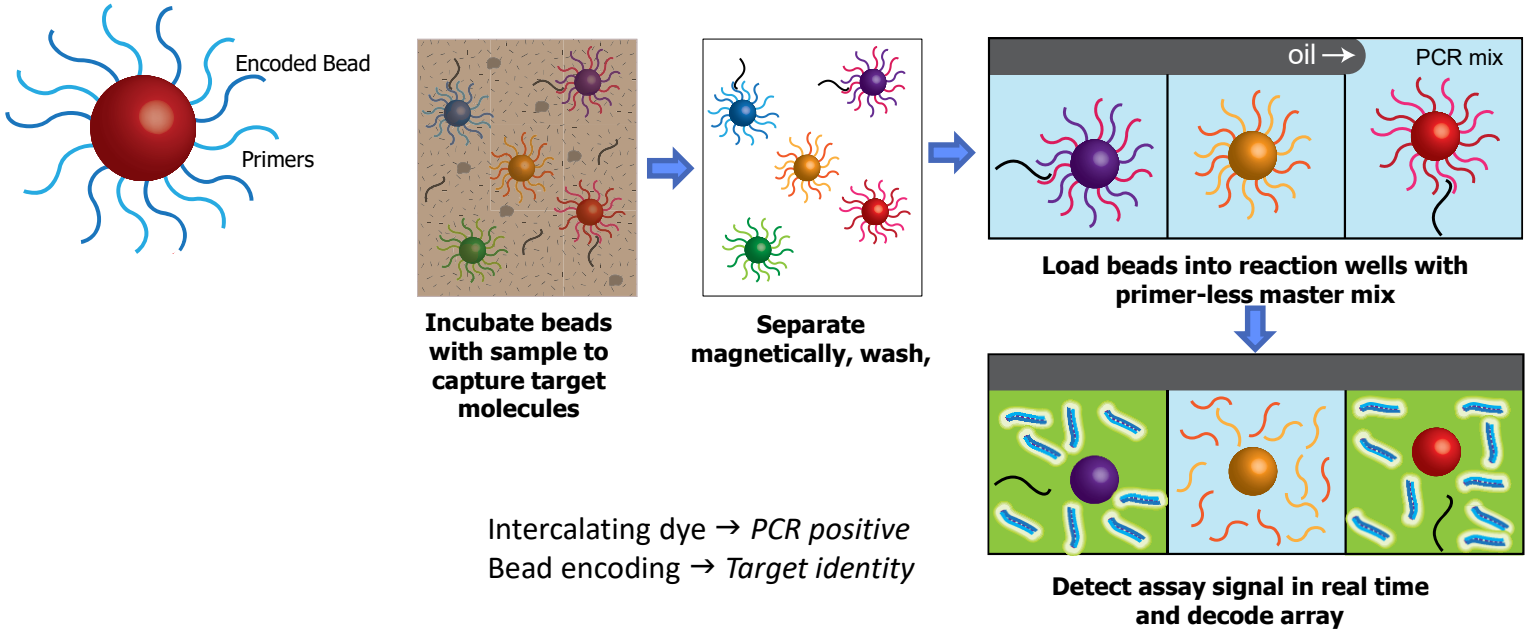
- Limited multiplexing for quantitative assays
- Complicated panel configuration

- Multiplexing while maintaining performance
- Non-Specific Binding (NSB)…

https://commons.wikimedia.org/wiki/File:Polymerase_chain_reaction.svg#/media/File:Polymerase_chain_reaction.svg

https://www.nordicbiosite.com/blog/elisa-principles-101

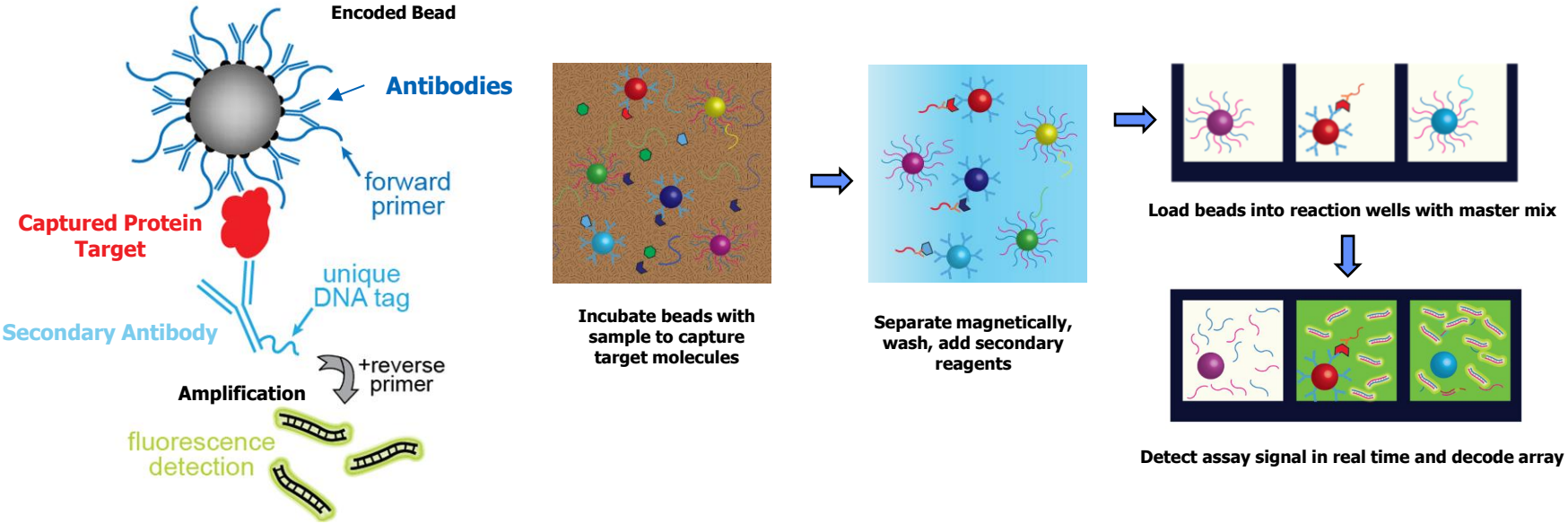
Singleplex NA Assays Multiplexed in Space



Intercalating dye → *PCR positive*
Bead encoding → *Target identity*

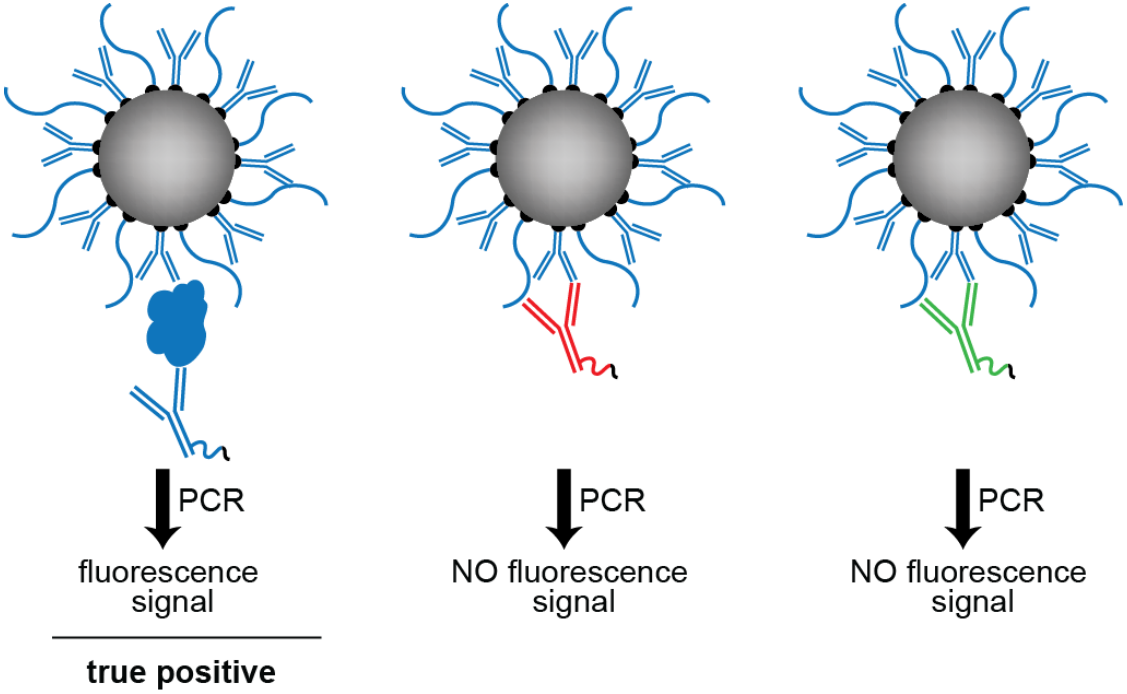
Seamlessly enabling dPCR, qPCR, and simplifying menu expansion.

Highly Multiplexed Proteins Assays on the Same Platform



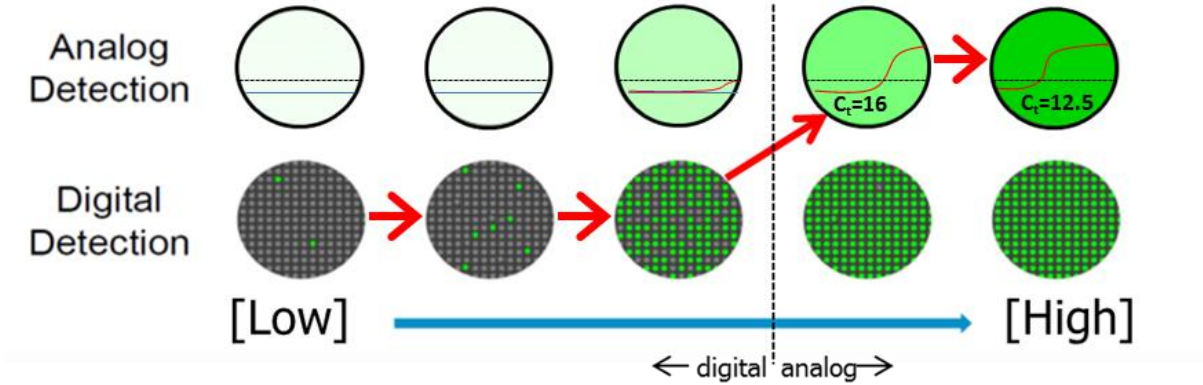
- **Unique iPCR assay strategy eliminates (cancels) signal from non-specific binding**
- **Allows performance to be maintained as plex levels increase**

Non-specific Binding Noise Canceling Strategy



The signal is like that in singleplex assays again!

Seamless Integration of Digital and Analog Quantification



Low Target Concentration

- ≤50% positive wells
- Digital quantitation

High Target Concentration

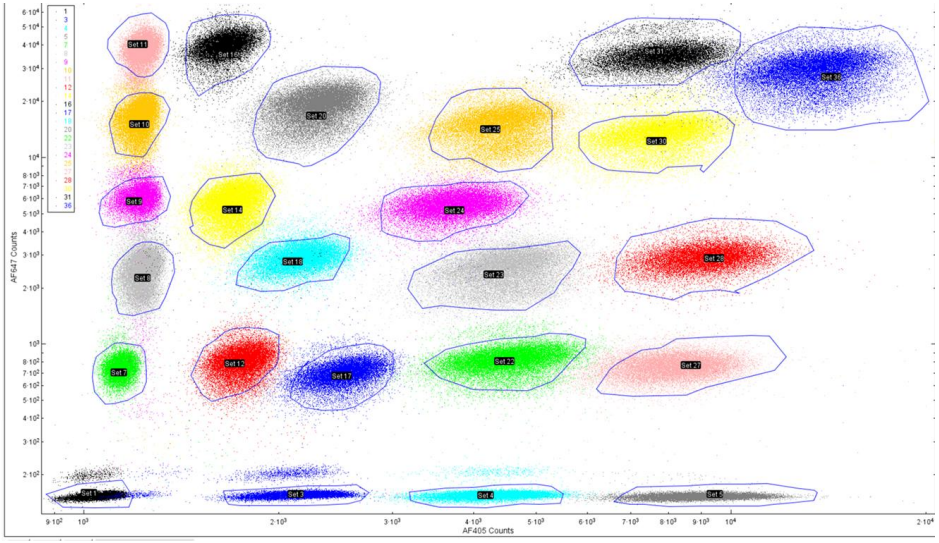
- All or most wells positive
- Use analog quantification, i.e. qPCR

Multiplex assays with up to 10^8 dynamic range from ***the same sample***

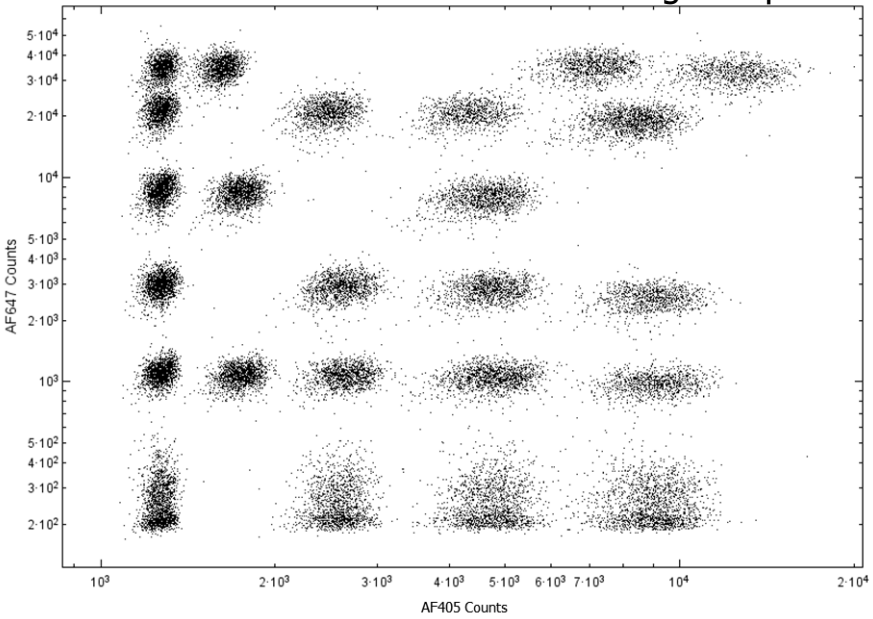
Encoded Bead Sets

- Selected the best 24 levels for larger scale synthesis (≈300M beads) each level

Targeted these 24 levels

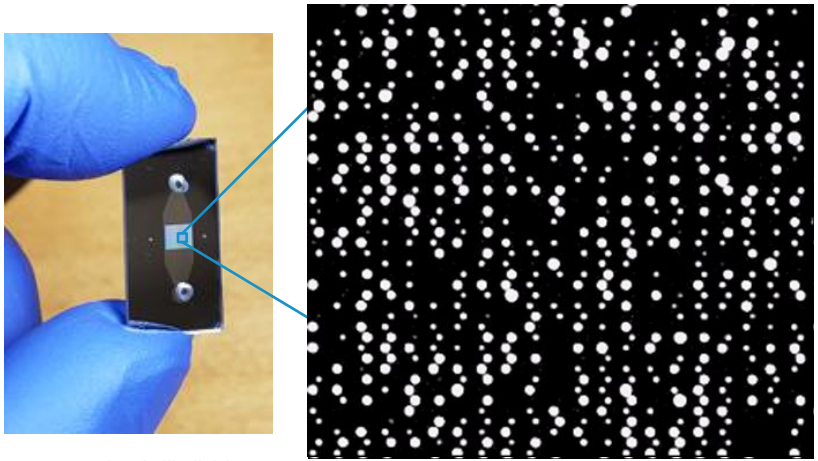


New beads measured in a single chip



Microwell Geometry

- Load a single bead
- Spatially separate assay signal from bead background
- Reaction volume ≈100 fL
- Fabricated using standard lithography processes

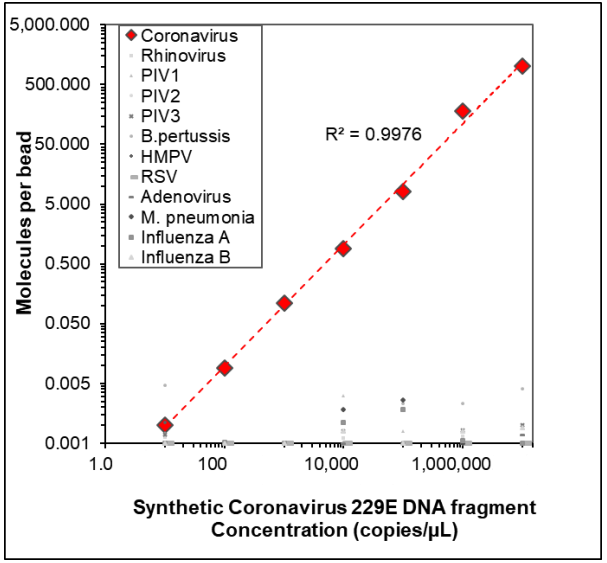


Proprietary well geometry enables high performance, multiplexing and quantification.

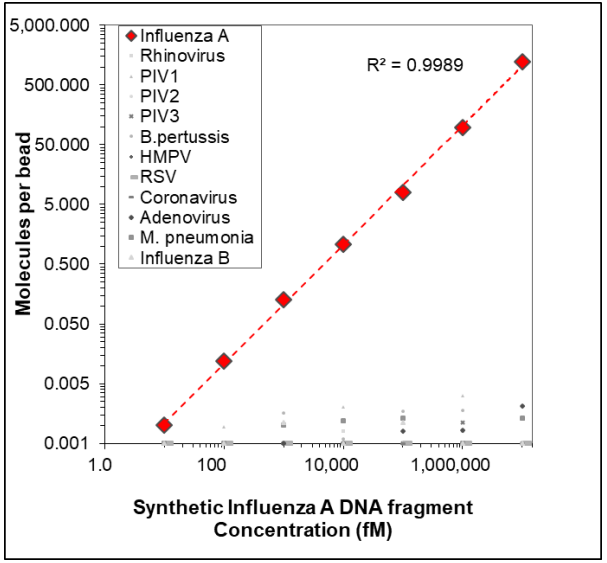
Linz, T. H.; Henley, W. H.; Ramsey, J. M., Lab Chip 2017, 17, 1076-1082.

daPCR 12-plex Calibration Curves

Coronavirus

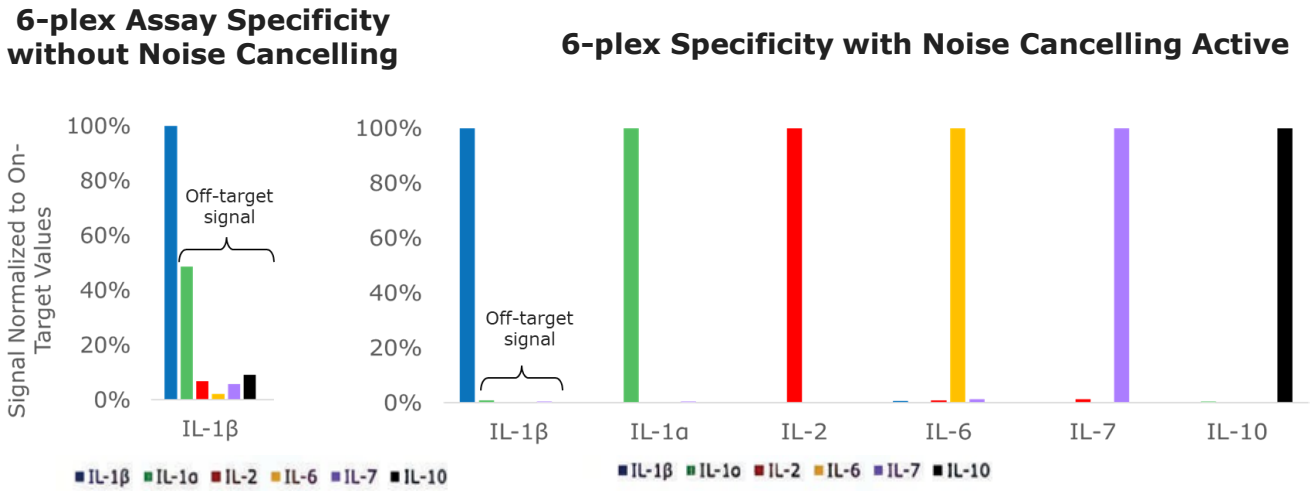


Influenza A



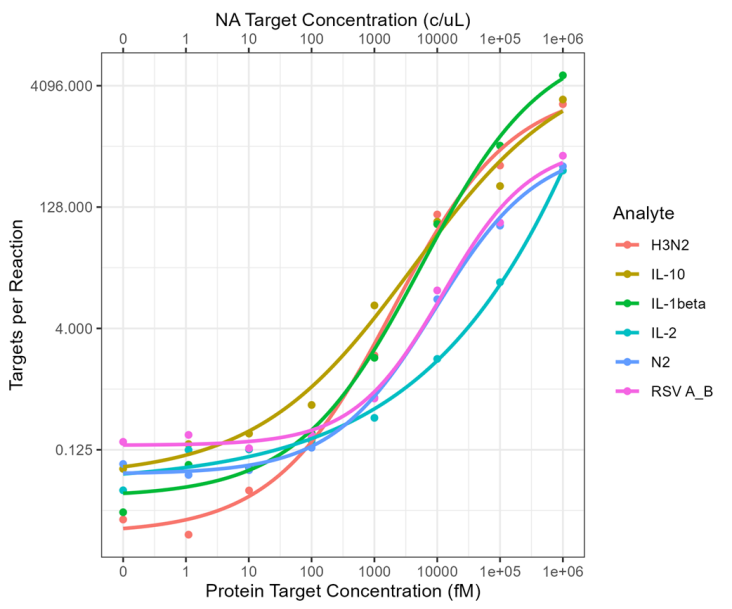
High sensitivity in multiplex, linear from 10 to 10 million copies/µL. Validation on a 12-plex panel, 100+ plex panels are possible with these LODs. 10 to 30-plex panels are possible with >10-fold lower LODs

ELIMINATION OF NON-SPECIFIC SIGNAL IN MULTIPLEX ASSAYS



- In this figure on the left, the only antigen present in this multiplexed assay is IL-1beta, however you can see that without noise canceling, significant false positive signal was generated for IL-1alpha beads, and to lesser degree for the other analytes in this target mix.
- On the right is data produced with noise cancelling turned on, and the same dose-response for IL-1beta now has a clean response with extremely low cross reactivity signal observed for other targets. Single antigen dose-response is depicted here for each target, showing typical background signal.
- Our modular design enables rapid reconfiguration of your multiplex panel without the need for re-optimization, offering unparalleled flexibility for your assay needs.

MULTIPLEX, MODULAR AND MULTI-OMIC



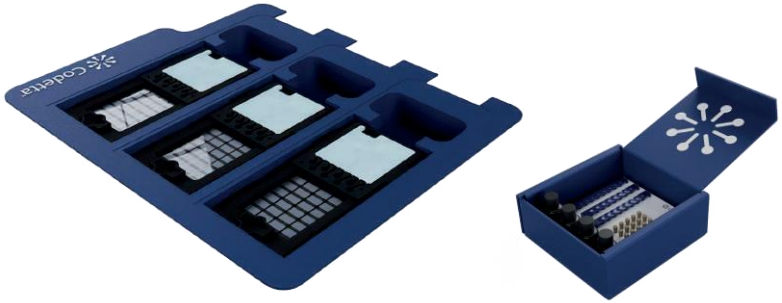
- Data were produced from a single human plasma sample with spike-in protein targets (cytokines) and NAs (respiratory pathogens).

- Nucleic acid concentrations are presented at the top of the plot, while cytokine concentrations are located at the bottom.

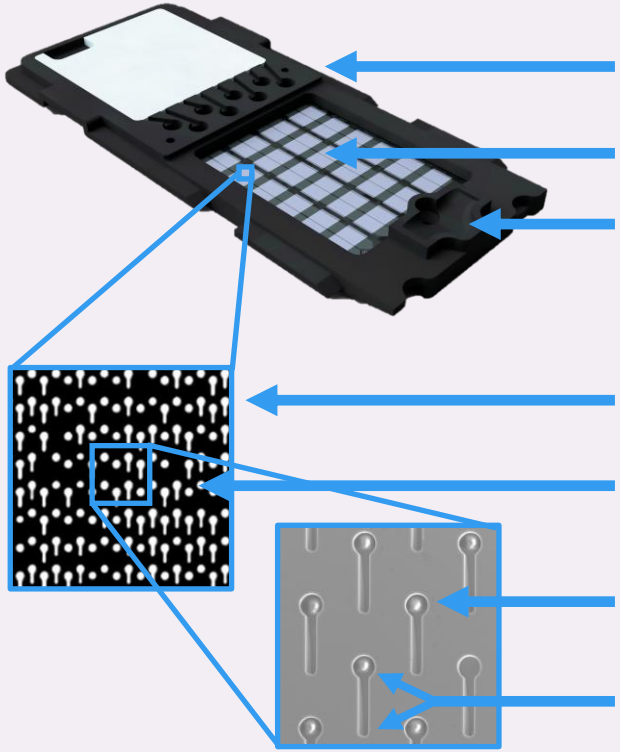
- By analyzing protein and nucleic acid data from the same sample, we enable correlations that were previously unattainable. This integrated approach opens doors to deeper understanding and groundbreaking discoveries.
- Our modular design allows you to fine-tune a single-plex assay and seamlessly plug-and-play additional plex, ensuring specificity remains uncompromised, even as plex increases.

INTRODUCING THE CODETTA BIO™ SUITE

Our **Codetta Bio Concerto System,** paired with **Prelude Kits** and **Prelude Chips**, represents the next generation of multi-omics, and will empower researchers to achieve comprehensive insights across multiple analytes with a single platform and workflow.



CODETTA BIO PRELUDE CHIPS



8 sample loading ports / chip

Glass layer with etched flow cells

Common reagent port

250,000 microwells etched into the silicon layer for each sample lane

PCR amplification of the DNA tag is measured with an intercalating dye

Each microwell can capture only a single bead

Microwell shape spatially separates bead decoding from analyte quantification

CODETTA BIO CONCERTO™ SYSTEM

Codetta Bio's Concerto instrument will revolutionize high-throughput, multiplex biomarker detection, combining digital PCR, real-time PCR, and immunoPCR
in a single, seamless workflow.

- Detects DNA, RNA and protein biomarkers in a single run
- Up to 20-plex
- Sub-pg/mL sensitivity and 8-log total dynamic range
- Ideal for biomarker validation and translational research
- Automated imaging, data analysis, and onboard quantification

Ramsey Group Members

(178)

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PONOR$

Microfabricated MS & CE

Defense Threat Reduction Agency (DTRA)
DARPA Microsystems Technology Office
National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL)
National Heart Lung and Blood Institute (NHLBI)
National Institute of General Medical Sciences (NIGMS)

Digital Assays

DTRA Diagnostics and Detection
University Cancer Research Fund
North Carolina Policy Collaboratory
NIH RADx-rad
UNC Center of Biomedical Microtechnologies
DARPA Biological Technologies Office

Technology Development Cycle

- It will never work!
- It works but is unimportant!
- There maybe some niche applications…
- It was a no-brainer from the start!

A variation on J.B.S. (Jack) Haldane quote, 1963