
Predicant Biosciences

201 Gateway Blvd

South San Francisco, CA 94080

Pete Foley: VP Engineering

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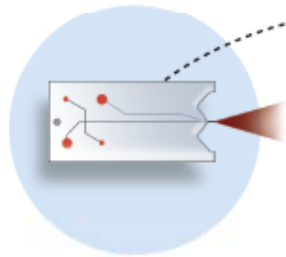


Who is Predicant?

Predicant Biosciences is a venture funded life sciences company based in South San Francisco that has developed an integrated platform for identifying and assaying protein biomarkers for clinical diagnostic use.

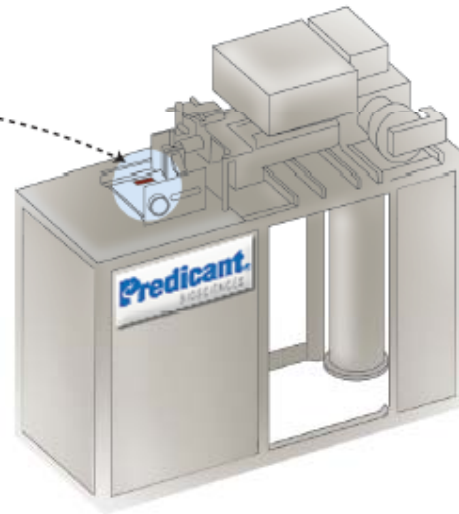
Integrated Discovery and Assay System

1. Separation



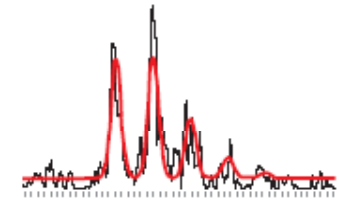
Microfluidics

2. Detection

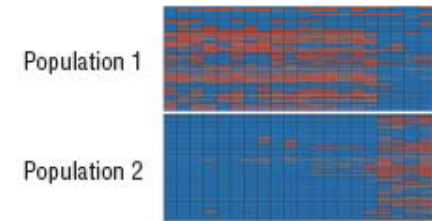


Mass Spectrometry

3. Pattern Analysis



Signal Processing



Pattern Classification

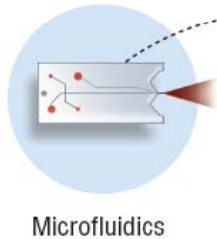
- Intact small proteins
- Proteome + Metabolome
- Disposable plastic chip
- Fast separations

- High resolution
- High sensitivity
- High mass accuracy
- High reproducibility/stability
- Interface optimized for chip

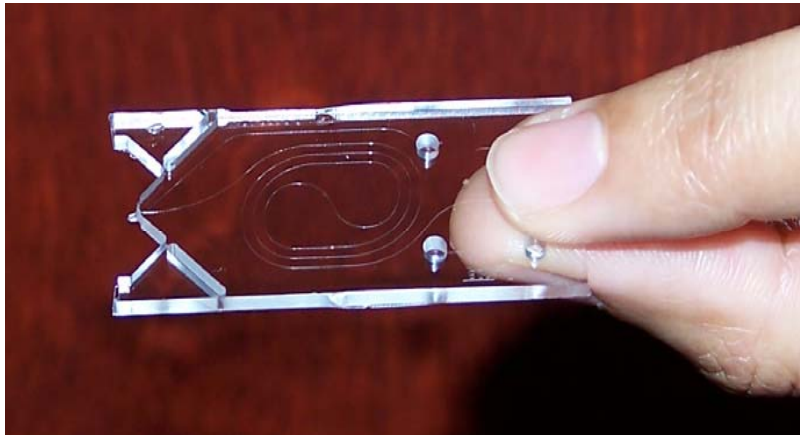
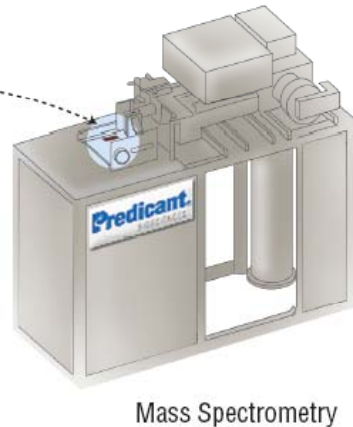
- Reproducible
- Designed for our platform
- Statistically based
- Subsequent protein ID

Microfluidic Chip CE-ESI-MS

1. Separation



2. Detection



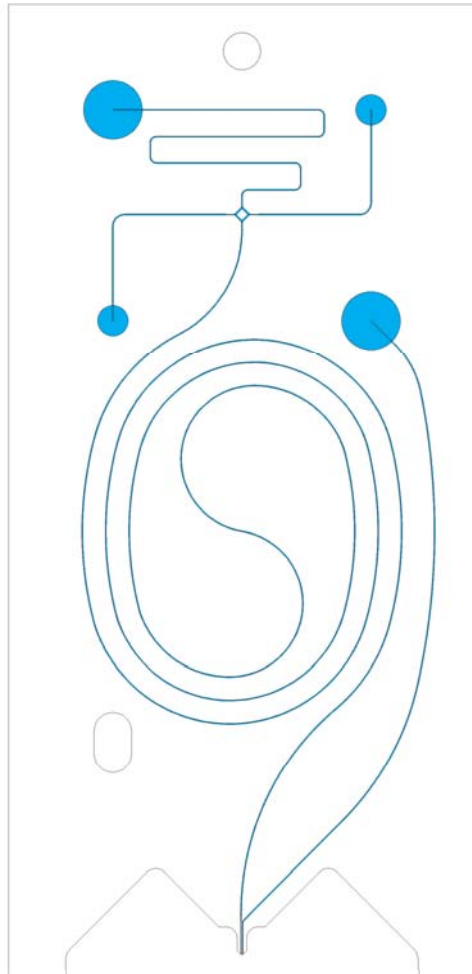
- Rapid electrophoretic separations (~12 minutes)
- Voltage driven injection and separations provide reproducibility and reliability
- Reproducible electrokinetic sample injection; low sample volume
- Recessed tip minimizes biohazard and fragility
- Single-use, plastic disposable – no carryover
- Separation channel coated to provide EOF and eliminate sample adsorption
- Integrated injection, separation, spray eliminate connections to give high reliability and reproducibility
- Multiple channels open onto the tip to provide electrical contact through solution rather than a tip electrode

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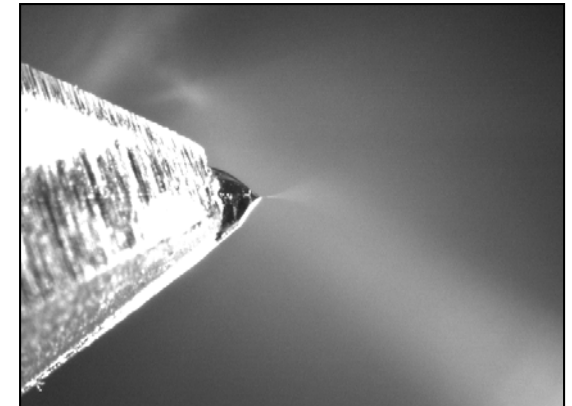
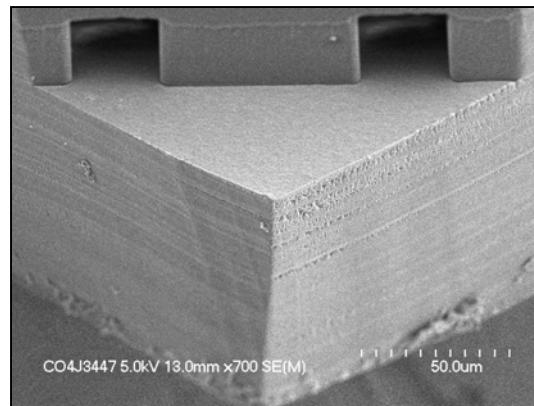
Microfluidic/CE Intellectual Property

- 3 Issued/Allowed Patents:
 - Microfluidic devices and methods for using devices containing protected ESI tips formed from the laminate layer with multiple channels that converge on the tip enabling electrical contact
- 7 Additional Patent Applications filed:
 - Covering alternate injection designs, surface coatings, fluid guiding features on the tip, electrical contact methods, and optimized designs for injection molded devices
- Licenses from:
 - Caliper: entire microfluidic patent portfolio for use in mass spec based biomarker discovery (non-exclusive).
 - Cornell: two patent applications covering microfluidic tips for electrospray ionization (Craighead) (exclusive)
 - Northeastern: one patent covering introduction of analytes from a microfluidic chip into a mass spectrometer (Karger) (non-exclusive)

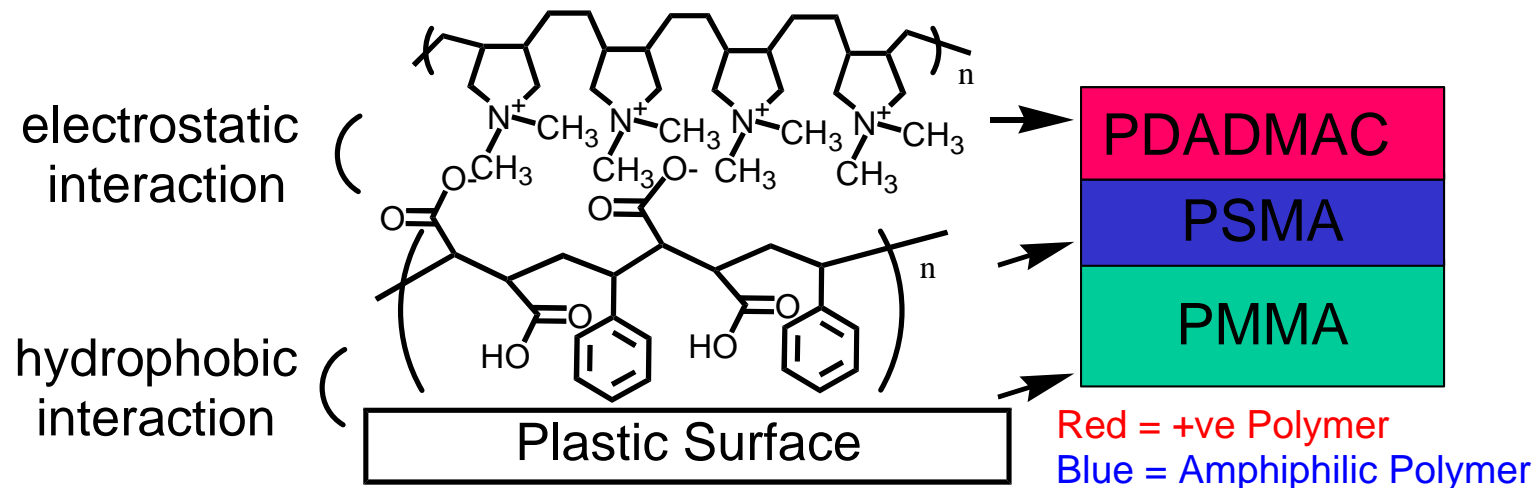
Channel and Tip Design



- **Diamond Injector**
 - Accurate sample injection
- **Separation channel**
 - Positively coated, electro-osmotic flow, < 100 nL/min
- **Side Channel**
 - Electrical contact channel
 - Uncoated
- **Spray voltage determined by voltage divider between right and left channels**

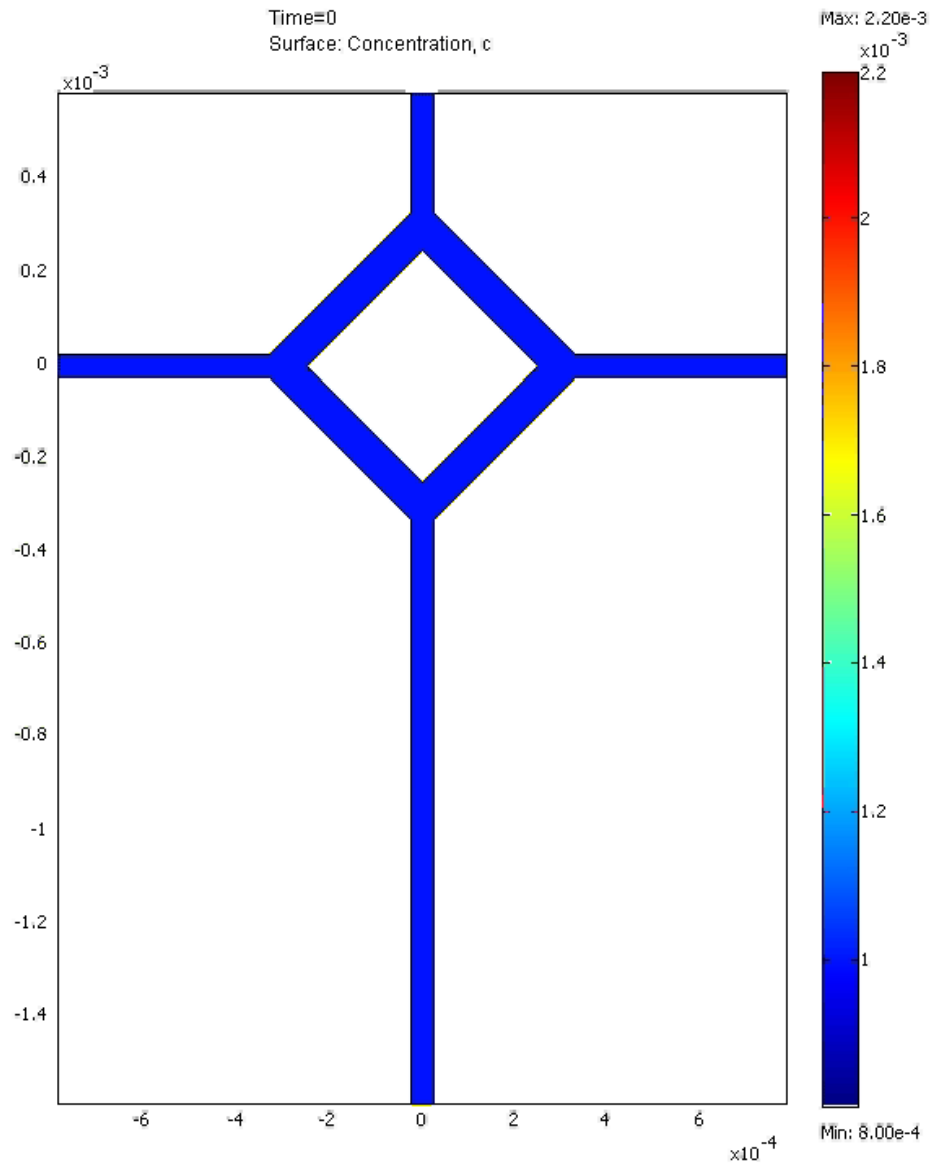


Microfluidic Channel Surface Coatings



- Simple coating process creating highly positive coating on channel surfaces at extremes of pH ranges
- Based on polymeric bilayer
 - Hydrophobic interaction to plastic chip
 - Electrostatic interaction between layer
- Stable to 50% organics, extreme pH and dry storage

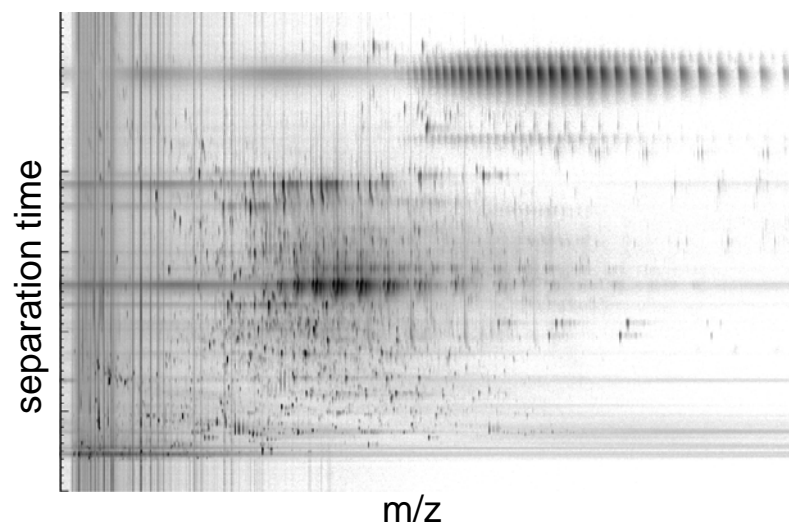
Diamond Injector for Sample Injection



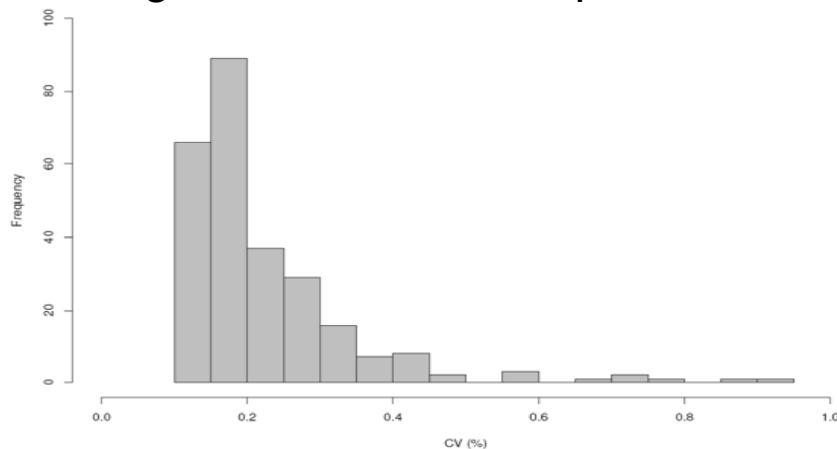
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Reproducibility and Sensitivity

2D-Gel Like Image of Typical Dataset



Histogram of Serum Component CVs

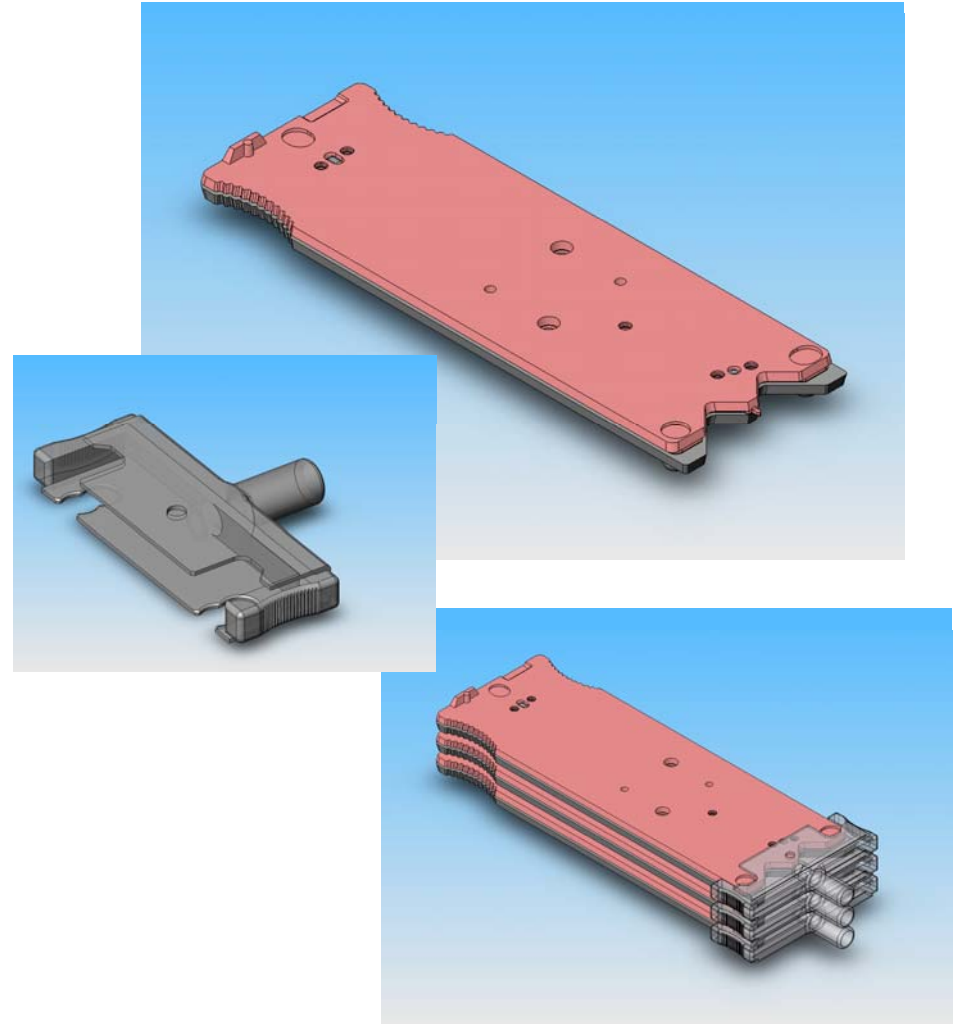


- N = 47 over 6 days
- CVs of serum components
 - 18% median, 25% average based on 300 serum components observed across all experiments
 - No trends observed
- Sensitivity
 - 2420 +/- 6% components observed
- Quantitative dynamic range
 - Over 3 orders of magnitude

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Path to Commercialization

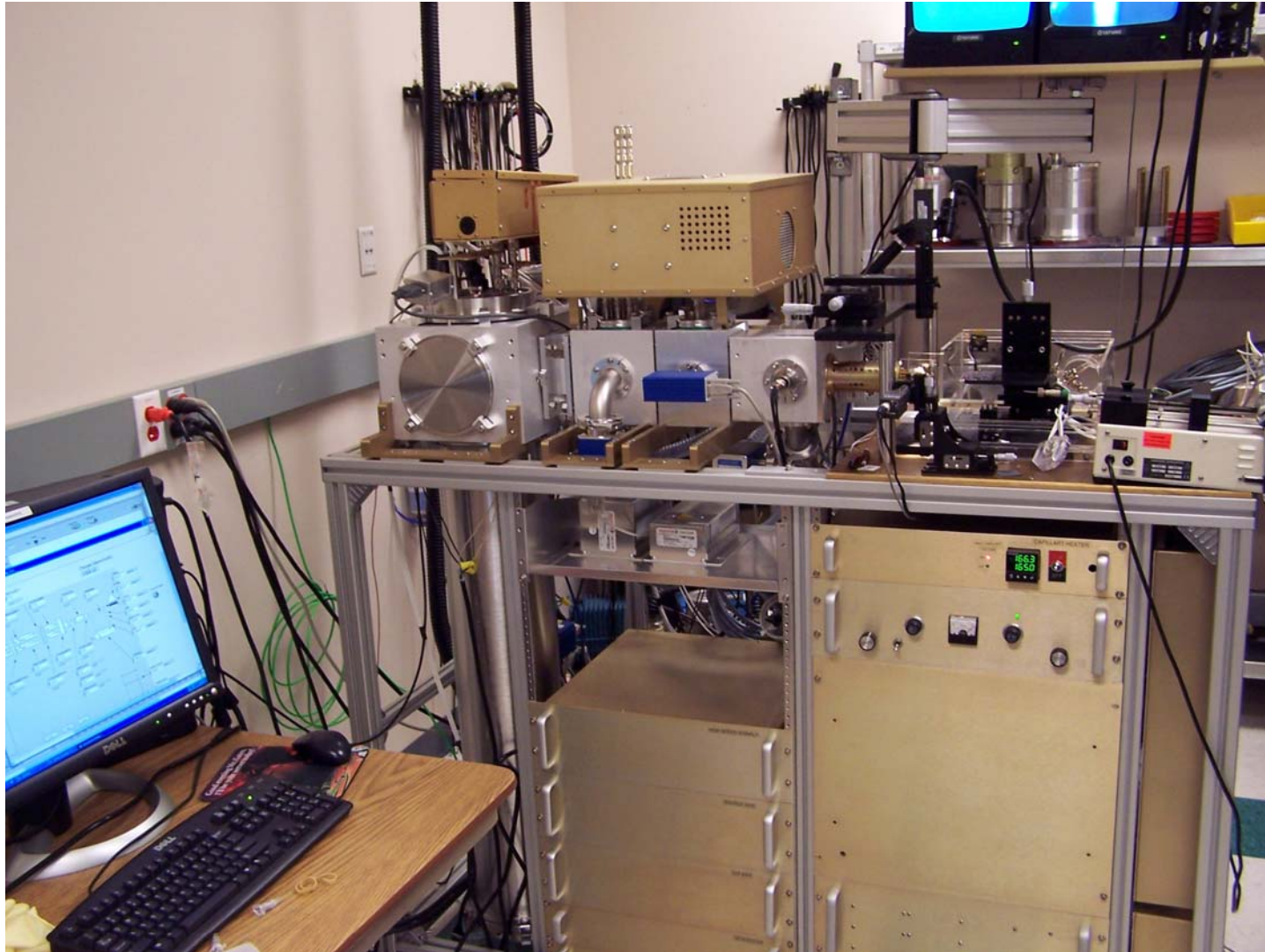
- Plastic for disposability
- Injection molded and compression bonded
 - Partnered with Weidmann Plastics
 - Part design complete, ready for tooling design
- Designed for manual or automated handling
 - Size of standard microscope slide (75x25 mm)
 - Features included to enable stacking chips
- Injection molded cap for ease of shipping, coating and assay
- < \$5/chip in modest volume



Current Chip Status

- Pilot manufacturing line exists at Predicant for compression molding, laminating, coating, and final inspection of chips.
- Current capacity is 5K/mo.
- We have used ~10K of these chips for discovery and validation studies.

ESI Orthogonal Time-of-Flight MS

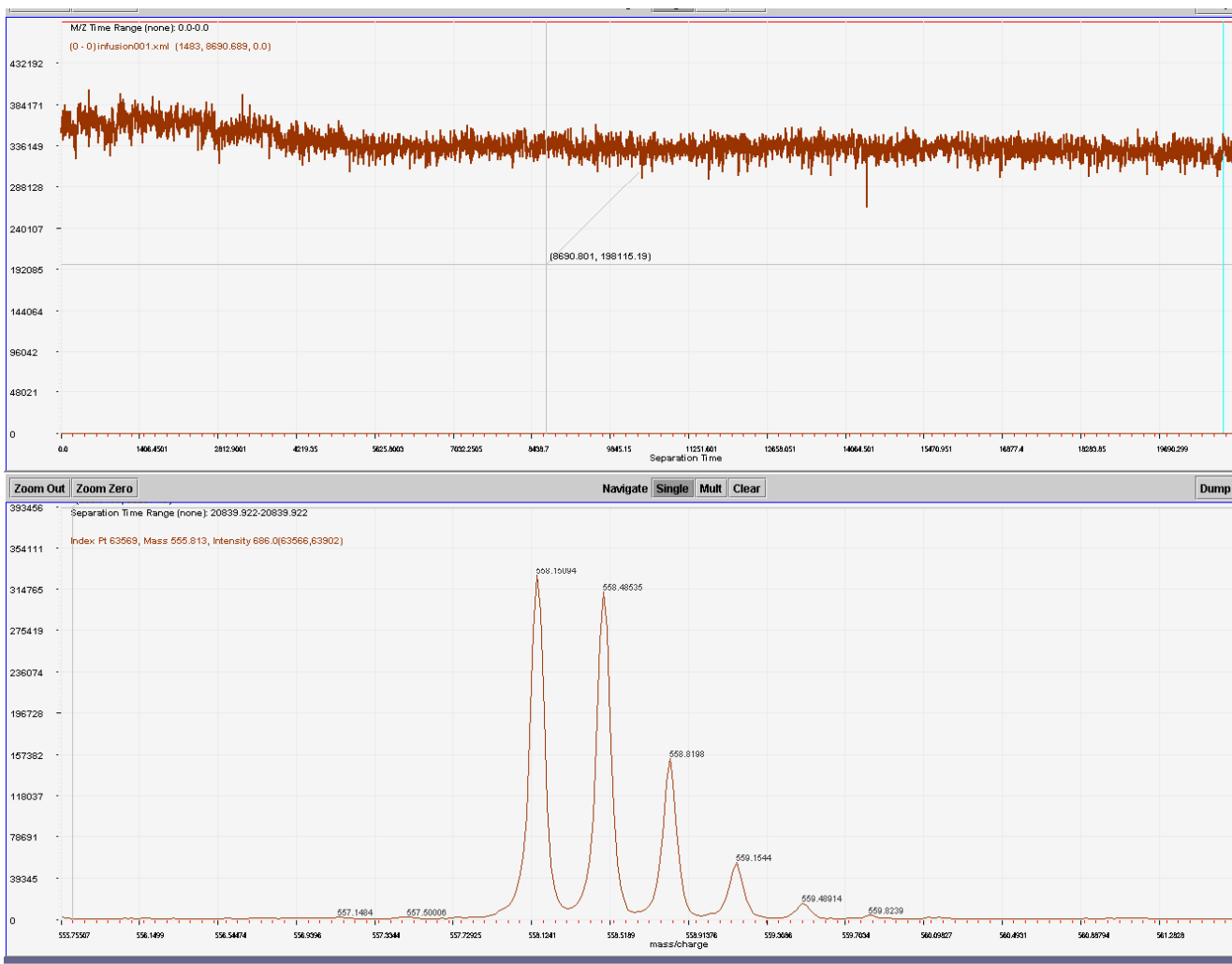


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Instrument Specifications

- Sensitivity
 - Sub-nanomolar (< fmol/uL)
- Mass Range
 - 200-5000 Da
- Resolution
 - 8K for Neurotensin 3+ (m/z 558) @ 2Ghz
- Mass accuracy
 - < 5ppm in internal calibration mode
- Dynamic Range
 - 10^4
- Stability
 - Intensity/resolution CVs < 3% for 6 hr infusion

Instrument Stability: 6hr. Infusion of 500nM neurotensin

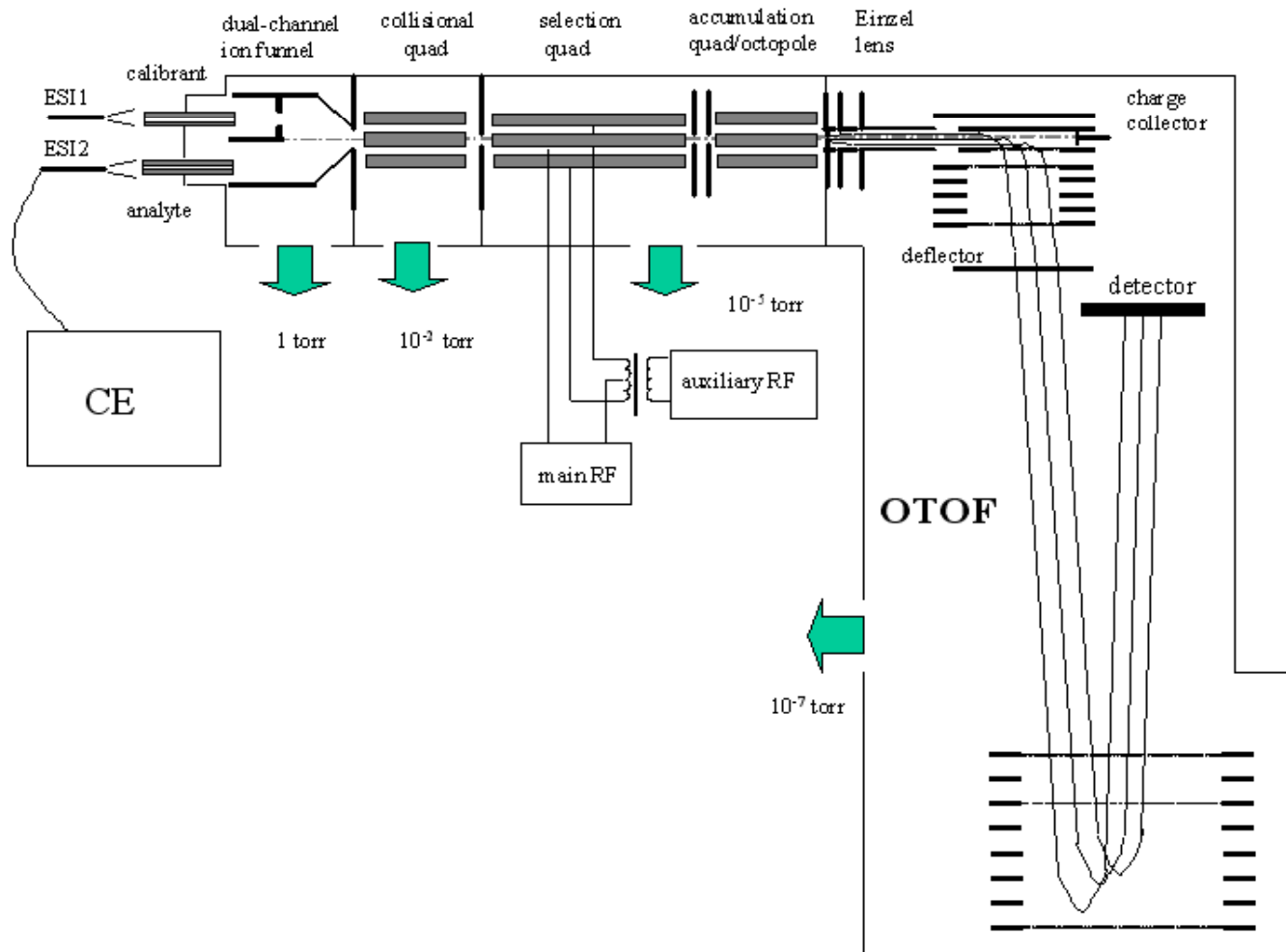


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Instrument Features

- Two spatially separated ESI sources
 - independent introduction of analytes and internal calibrants
- Dual channel electrodynamic ion funnel
- 2Ghz 8-bit acquisition system
- 40mm bipolar detector
- Proprietary quadrupole RF drive
- Spray visualization system
- Real-time software logging of all instrument parameters

Instrument Block Diagram



Instrument Advanced Features

- Mass Selection
 - Data dependent ion selection based on resonant excitation and ejection for improved sensitivity and dynamic range.
 - Arbitrary filter structure with resolution 300
- Trapping
 - Linear ion trap to accumulate data dependently selected ions for increased sensitivity and dynamic range

Instrument Control Screen Shot

The screenshot displays the 'LinearSectionWithReadBackVoltControlDlg.vi' control window. The main area features a schematic of the instrument's linear section with various voltage controls. The 'ChargeCollectorVoltIn' is set to $5.47E-12$. The 'Pressure' section shows 'TOF Pressure Enable' is active, with 'Funnel' at 0.0901, 'Collision Quadrupole' at 0.000101, 'Selection Quadrupole' at $2.01E-6$, and 'Flight Tube' at $1.04E-6$. The 'Errors' section shows a yellow warning: 'Out of range. The input signal you are measuring has gone out of the specified measurement range. Check the signal'. Below this, 'Pusher Error' and 'Puller Error' are listed with green indicator lights.

Power Supplies

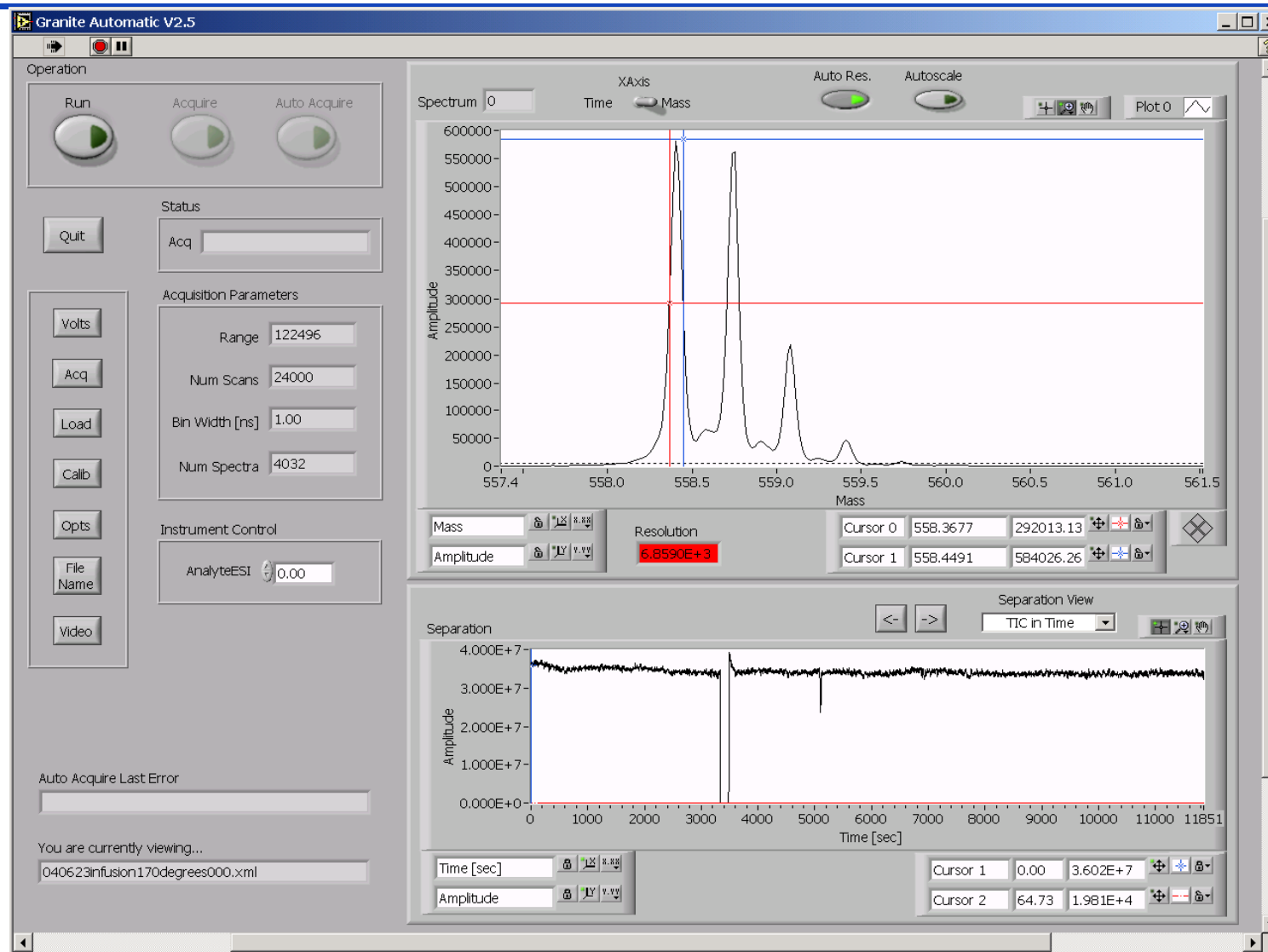
PA140PosHighVolts	MA17NegHighVolts
295.83	-108.19
PA140NegHighVolts	MA17PosHighVolts
-26.96	107.97

Control Parameters

DisruptorPlate	0	CollQuadBias	0.00	SelectQuadExit	0.00	InletLens	0.00
FunnelEntrance	0.00	PreselectQuadBias	0.00	AccumQuadBias	0.00	TransferTube	0.00
FunnelExit	0.00	AccumQuadExit	0.01	TransferTubeEx	0.00	PMTBias	-2.00
CalibrantESI	0.00	SelectQuadBias	0.00	AccumQuadEntrance	0.00	MCPBias	-7.00
AnalyteESI	0.00	SelectQuadEntrance	0.00	DeflectorLens	0.00	Pusher	0.00
InletCapillaries	0.00	AccumQuadExit	0.00	DeflectorLensA	0.00	Puller	0.00
FunnelGate	0.00	DeflectorLensB	0.00	TOFDeflectorX	0.00	AccelEntrance	-40.00
InletCapillaries	1.11	TOFDeflectorY	-0.10	Liner	-20.00	ReflectronDecel	-3.00
FunnelGate	-0.12	ReflectronBack	5.00				

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Instrument Control SW – Spectrum Viewing



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Predicant Reference Lab



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Instrument Physical and Cost

- No external gasses or water required
- 120V only
- Material cost: ~\$120K in prototype qtys
 - Major cost items:
 - Turbopumps (2) and backing pumps (3)
 - 40mm bipolar detector
 - 2 Ghz 8-bit ADC acquisition PCI board
 - National Instruments Fieldpoint control modules

Mass Spectrometry IP

- Patents
 - Multiplexed Orthogonal Time-of-Flight Mass Spectrometer (US 6,900,431)
 - A-Priori Biomarker Knowledge Based Mass Filtering for Enhanced Biomarker Detection (US 6,958,473)
 - Pending Patents:
 - Scan Pipelining for Sensitivity Improvement of Orthogonal Time-of-Flight Mass Spectrometers
 - **Field Termination Grid in the Ion Extraction Region of an Orthogonal Time of Flight Mass Spectrometer**
- Licenses
 - Non-exclusive license to ion funnel (PNNL)
 - Non-exclusive license to dual ion funnel (PNNL)
 - But exclusive for TOF
 - Exclusive license to DREAMS (PNNL) for TOF
 - Rights to transfer all of the above licenses (with some restrictions)

Contact Information

- Predicant Biosciences IP
 - Pete Foley, VP Engineering
 - 650-814-7710
 - pfoley@predicant.com