Continuing the Quest for a Mass Spectrometry-Based Plate Reader: Evaluating Laser Diode Thermal Desorption (LDTD) coupled with Nanoliter Dispensing for HT-ADME and Other HTS Applications

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Outline

- Role of mass spectrometry (MS)-based high-throughput (HT) screening in early drug discovery
- Advantages of MS-based analysis
- Quick overview of current front-end automation tools used to improve MS-based throughput
- Continuing the quest for MS-based, sub-second sample readout speeds for HT-ADME and HTS support
  - Laser Diode Thermal Desorption (LDTD) evaluation and potential uses in early drug discovery
- Summary and next steps

HT MS-based Screening in Early Drug Discovery

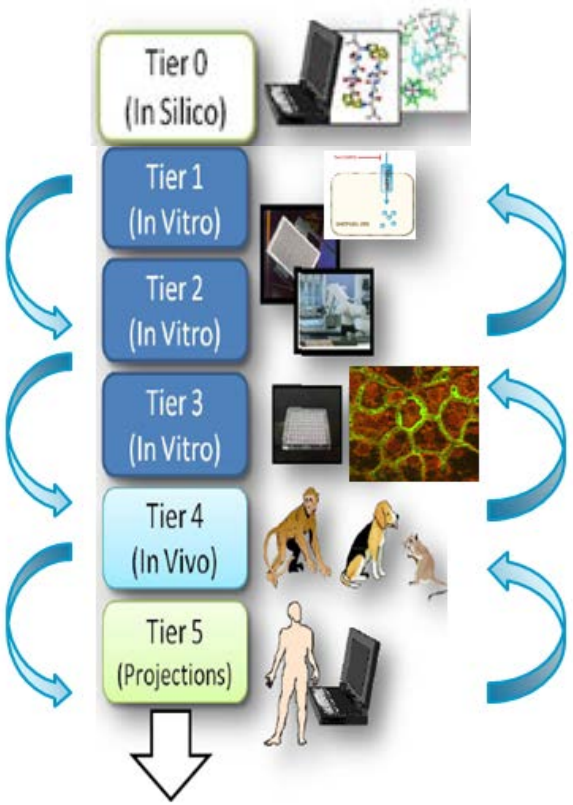
Maximize availability and impact of *in vitro* screening data to drive informed decision-making throughout discovery & development

Commonly performed MS-based screening applications

- ***In vitro* liability screening (HT-ADME)**
  - 1000s of samples to be analyzed daily
  - Characterize PK and Toxicity of NMEs
    - Assess potential liabilities
    - Selecting/prioritizing NMEs for advancement

Other potential screening applications

- **Biological activity screening (HTS)**
  - >10,000 samples to be analyzed daily
  - Miniaturization of assay format necessary

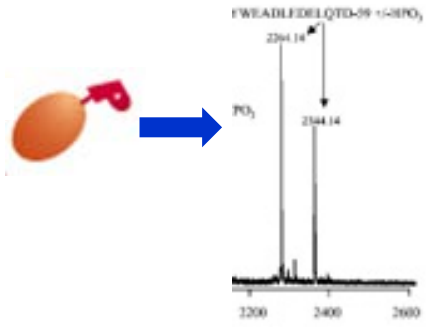


Advantages of MS-based, "Label-Free" Screening

- Allows us to use clinically-relevant, native probe substrates instead of molecular labels, fluorescent dyes, radiolabeled probes, etc…
  - May reduce cost
  - Eliminates radioactive waste streams
  - More predictive assay suites
    - Better *in vitro-in vivo* correlation
- Flexible and sensitive platform
- Able to quantify multiple analytes simultaneously

Replacing this:

With this:



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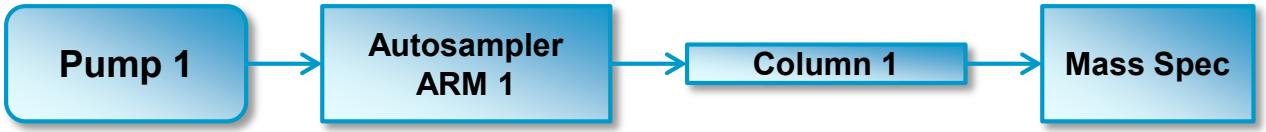
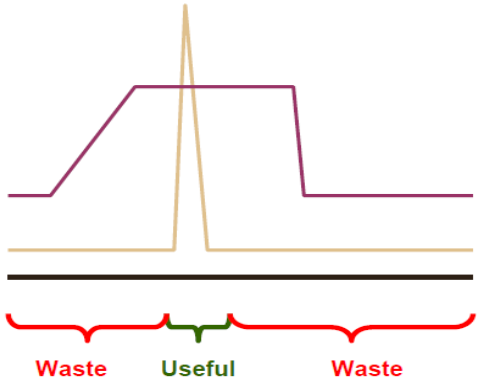
Supporting HT-ADME & HTS: Traditional LC-MS/MS

- Samples acquired in sequential manner (Slow - minutes per sample)
- Not possible to support high volumes associated with HT-ADME or HTS.





- MS detector idle most of time

HT-ADME 5000 Samples	×	1.5 Minutes Per Sample	=	125 Hours 5 Days!
HTS 50,000 Samples	×	1.5 Minutes Per Sample	=	1250 Hours 52 Days!

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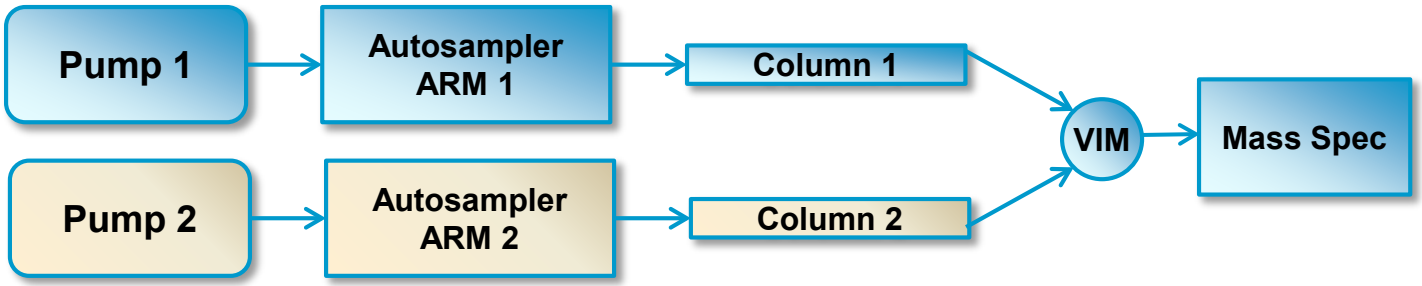
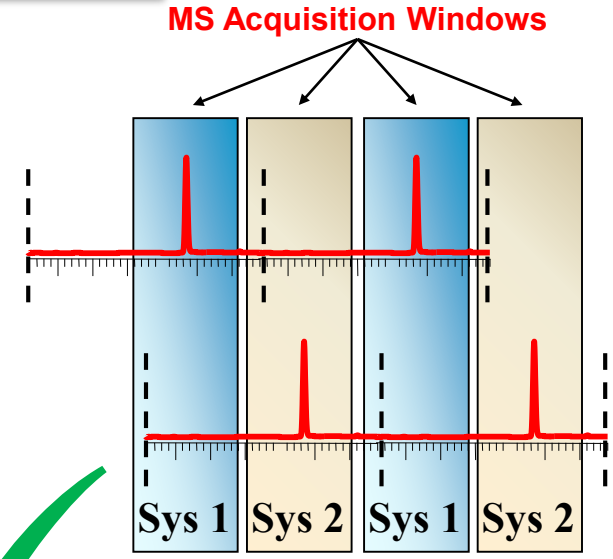
Supporting HT-ADME & HTS: Multiplexed LC-MS/MS

Thermo Cohesive ARIA System, ADDA System (Apricot Designs), etc.





Increased Throughput (25 - 45 seconds/sample):

- Staggered, parallel operation of multiple channels on single MS system
- Staggered injections / alternating MS detection
- Reduce MS idle time by only acquiring relevant data

HT-ADME 5000 Samples	×	25 Seconds Per Sample	= 34 Hours 1.5 Days ✓
HTS 50,000 Samples	×	25 Seconds Per Sample	= 350 Hours 15 Days! ✗

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Supporting HT-ADME & HTS: On-line SPE-MS/MS

RapidFire™ System (Agilent) or ADDA System (Apricot Designs)

- **Significant throughput gains over LC-MS/MS**
  - **~10-15 seconds per sample**
- **High-speed on-line solid phase extraction (SPE)**
  - **"Trap and Elute"**
  - **No Chromatography**
  - **Amenable to substrate-based (probe-specific) assays**
    - **CYP Inhibition, Transporter Inhibition, etc.**

HT-ADME **5000 Samples**	×	**10 Seconds** **Per Sample**	=	**14 Hours** **<1 Day** ✓
HTS **50,000 Samples**	×	**10 Seconds** **Per Sample**	=	**140 Hours** **6 Days** ✗

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Emerging Technologies: Laser Desorption Ionization

"Next-Generation" Methodologies

Laser Desorption Ionization (LDI) Techniques

- Faster sample readout & smaller volume requirements
- Direct analysis (no LC or SPE, no mobile phase)
  - *Liquid samples deposited directly onto plate*
- Ideal for "probe-specific" assays
- Throughput speeds approaching or equal to plate-reader assays

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MALDI-TOF

Bruker Corp. (www.bruker.com)

LDTD-MS/MS

Phytronix (www.phytronix.com)

Quick Comparison: MALDI-TOF vs. LDTD-MS/MS

Matrix-assisted laser desorption/ionization (MALDI) - time-of-flight (TOF)

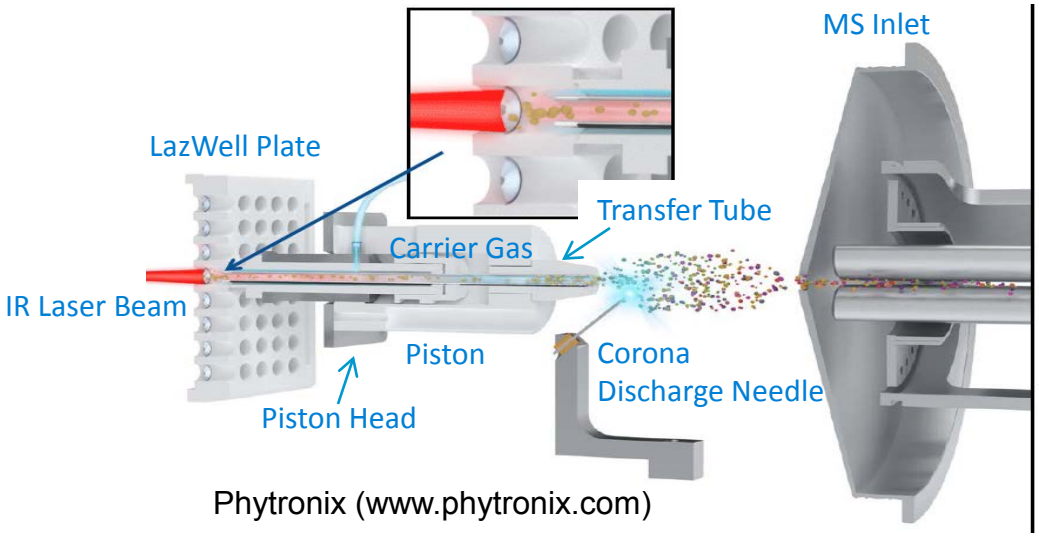
- Small molecule targets can "get lost" in biological background and MALDI matrices
  - Susceptible to ionization suppression effect which reduces S:N
- TOF analyzer collects data on wide range of ions
  - Potential for simple method development
  - Generally lower sensitivity for quantitation

Laser Diode Thermal Desorption (LDTD)-Tandem Mass Spec (MS/MS)

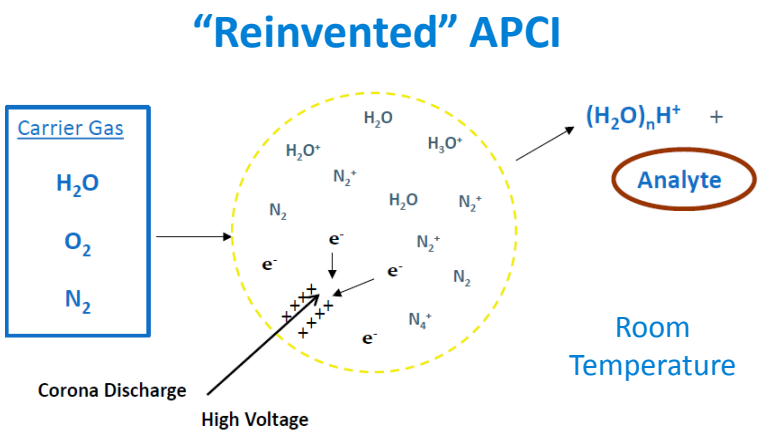
- APCI with Triple Quadrupole analyzer less susceptible to ionization suppression
  - Selected Reaction Monitoring (SRM) mode
    - Requires up front method development
    - Typically better sensitivity (background reduced significantly)
      - Gold standard for quantitation by MS

LDTD – Quick Overview of Process

Combines ultra-fast thermal desorption with efficient gas-phase APCI



Phytronix (www.phytronix.com)



- **Samples deposited into well of stainless steel LazWell plate and evaporate into analyte-nanocrystal structure**
- **Infrared laser diode heats back of well to produce thermal desorption**
- **Neutral gas-phase molecules enter the piston**
- **Neutrals enter corona discharge region to undergo APCI**
- **"Reinvented" APCI: no solvent, no mobile phase, only water as source of protons**

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HT-ADME Evaluation – CYP Inhibition Assay

Goal:

- **Couple acoustic sample deposition (ASD) with LDTD analysis**

Determine:

- **Optimal laser pattern for speed/reproducibility**

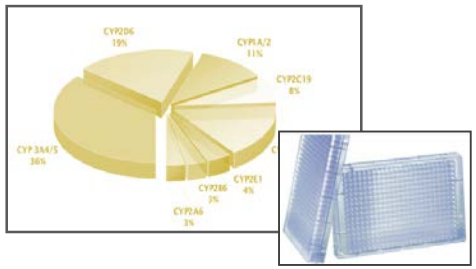
Demonstrate:

- **Adequate bioanalytical performance**
- **Throughput much faster than current production method (RapidFire)**
  - **Discrete analysis of individual CYP isozyme-metabolite pairs**
  - **Potential of sample multiplexing (reduce consumables cost/increase speed)**

Desired Workflow: Coupling ASD with LDTD-MS/MS

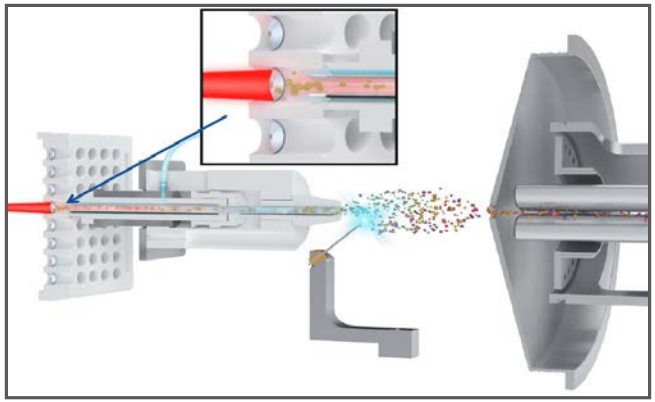
Couple Acoustic Sample Deposition (ASD) with LDTD MS to achieve High Throughput MS readout (ASD-HTMS)

2. Transfer nL volumes onto a stainless-steel LazWell 384 plate



1. Sample incubation in Echo-qualified 384-w plates

3. LDTD ion source (Phytronix) attaches to front of Sciex MS



Sample readout from 2-6 seconds/well

1. "Acoustic Sample Deposition Coupled With LDTD-MS/MS Takes High-Throughput MS to the Next Level," Z. Haarhoff, A. Wagner, T. Zvyaga, W. Shou, P. Picard. 4th SLAS Annual Conference & Exhibition, **2015**, Washington, DC.

2. Haarhoff, Z., Wagner, A., Picard, P., Drexler, D. M., Zvyaga, T., & Shou, W. (2015). Coupling Laser Diode Thermal Desorption with Acoustic Sample Deposition to Improve Throughput of Mass Spectrometry–Based Screening. *Journal of biomolecular screening*, 1087057115607184.

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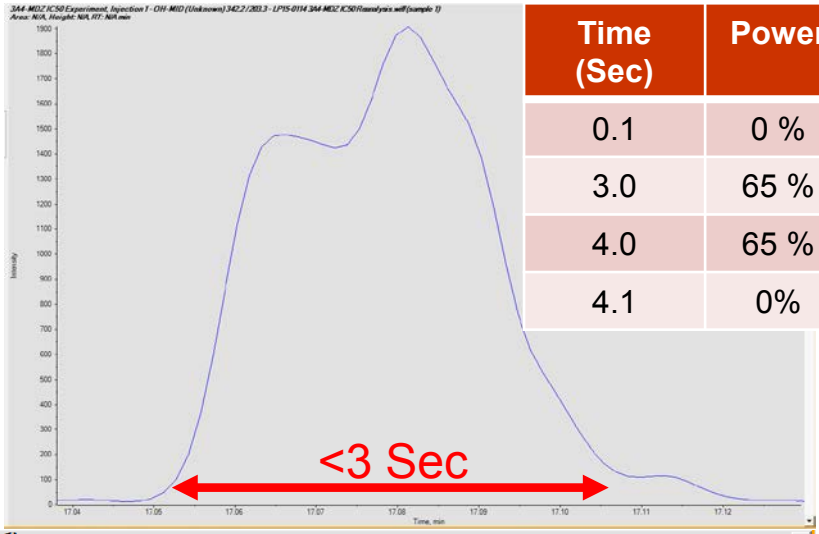
Determine Optimal/Fastest Laser Pattern

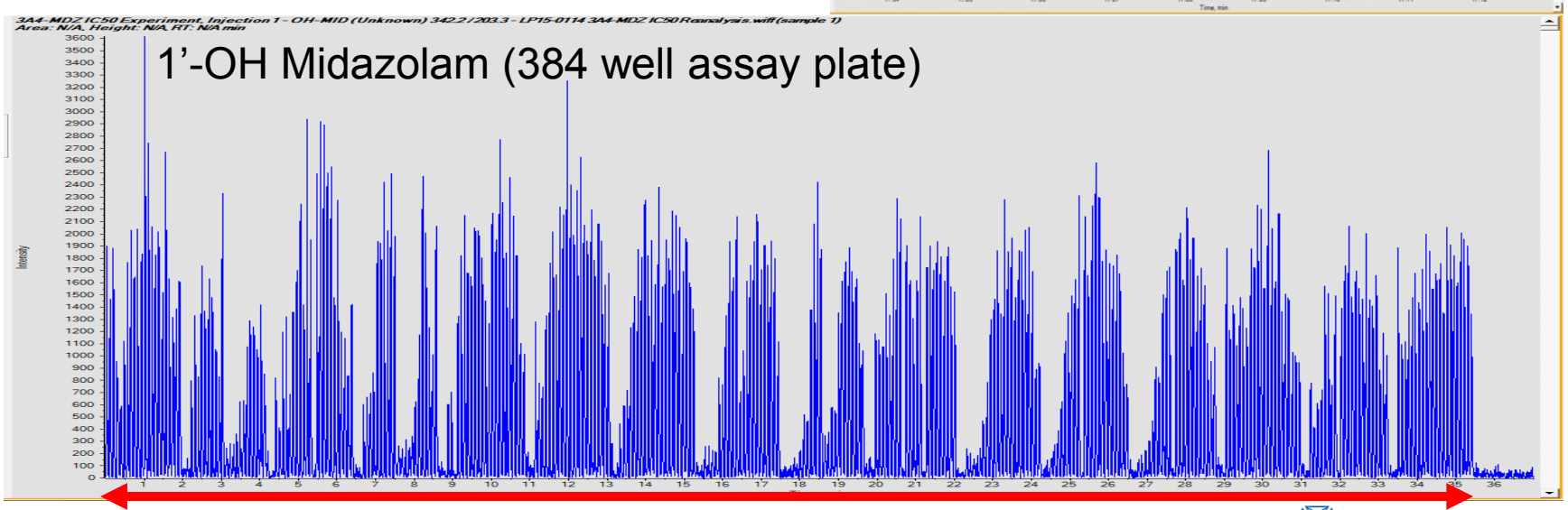
Importance of MS Scan speeds

Sciex API4000

- Slow cycle times per transition
- Laser pattern had to be lengthened for optimal results
  - 36 minutes per 384 well plate
  - Poor peak shape



Time (Sec)	Power
0.1	0 %
3.0	65 %
4.0	65 %
4.1	0%



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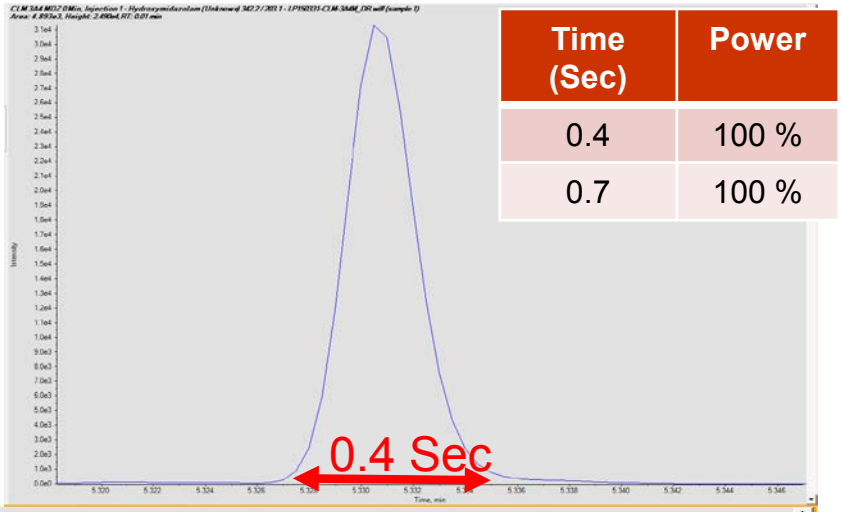
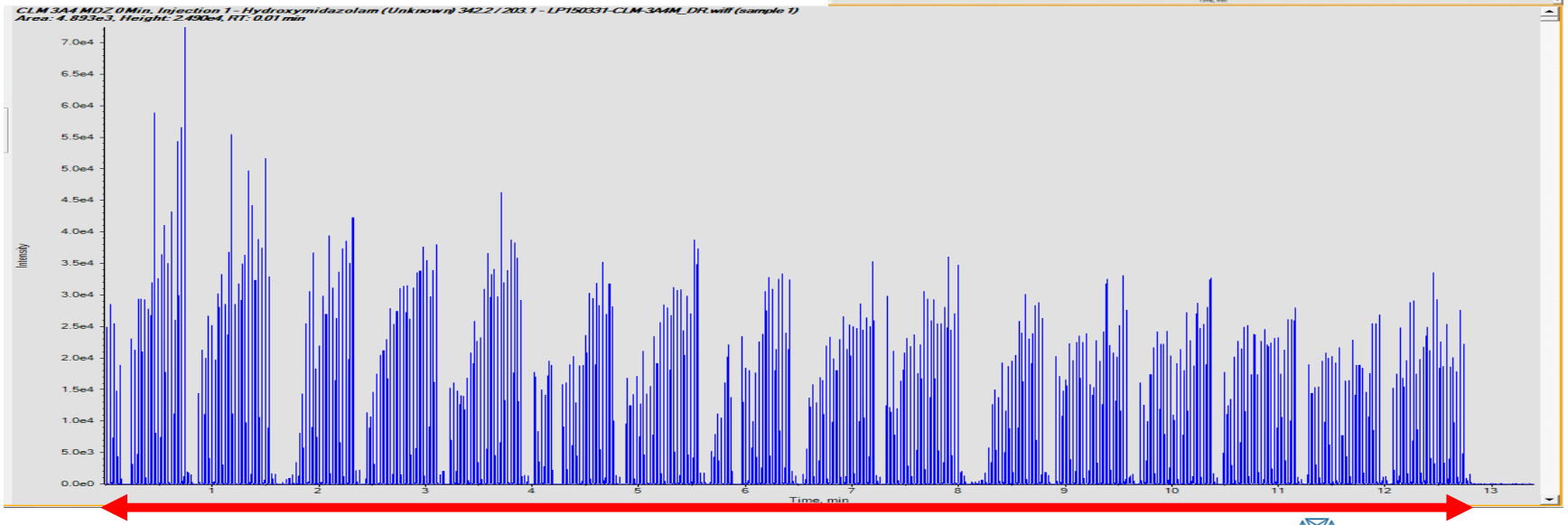
Determine Optimal/Fastest Laser Pattern

Sciex 4500 TripleQuad

- Faster scan speeds (reduced to 5ms per transition) allowed us to increase speed of laser pattern
- Maximized throughput
  - 13 minutes per 384 well plate
  - Sharp peaks – better signal



Time (Sec)	Power
0.4	100 %
0.7	100 %



13 Minutes – **5x** faster than RF

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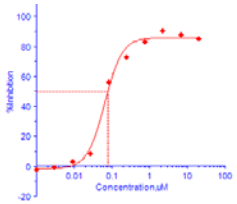
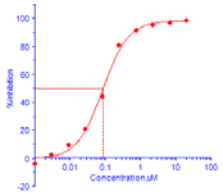
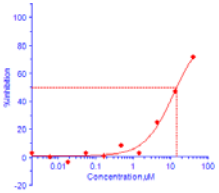
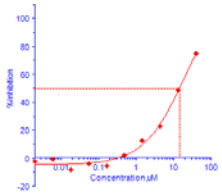
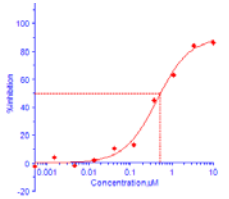
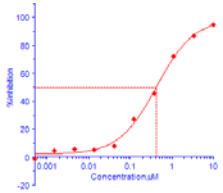
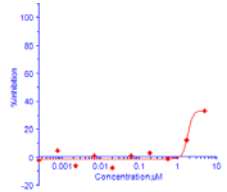
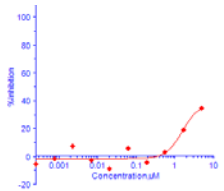
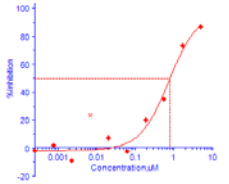
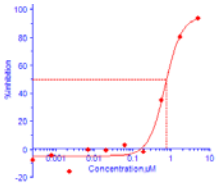
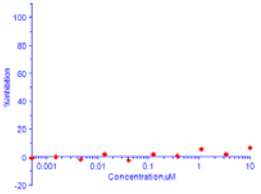
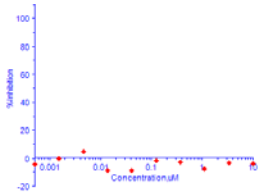
CYP2C9 Assay Controls: LDTD vs. RapidFire™

LDTD	RapidFire™	LDTD	RapidFire™
Tienilic Acid  IC50 = 0.079 µM	Tienilic Acid  IC50 = 0.088 µM	Fluvoxamine  IC50 = 14.6 µM	Fluvoxamine  IC50 = 14.4 µM
Sulfaphenazole  IC50 = 0.53 µM	Sulfaphenazole  IC50 = 0.42 µM	Ketoconazole  IC50 = > 5 µM	Ketoconazole  IC50 = > 5 µM
Clotrimazole  IC50 = 0.79 µM	Clotrimazole  IC50 = 0.76 µM	Furafylline  IC50= > 10 µM	Furafylline  IC50= > 10 µM

"Acoustic Sample Deposition Coupled With LDTD-MS/MS Takes High-Throughput MS to the Next Level," Z. Haarhoff, A. Wagner, T. Zvyaga, W. Shou, P. Picard. 4th SLAS Annual Conference & Exhibition, **2015**, Washington, DC.

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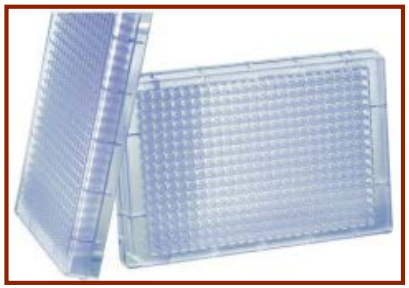
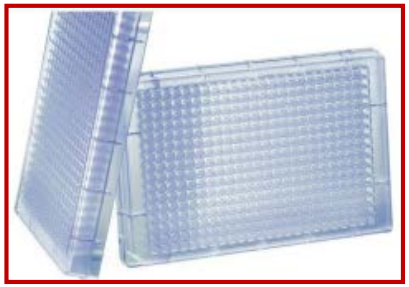
Post-Reaction Multiplexing: ASD with LDTD-MS/MS

3x reduction in # of plates to analyze with LDTD-MS/MS

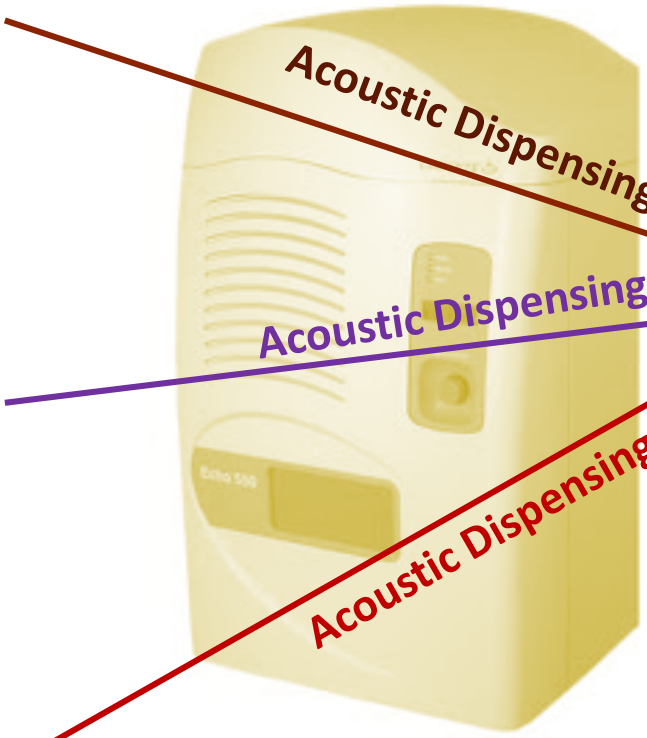


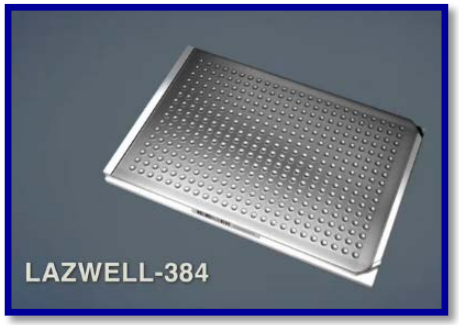
3A4

2D6



2C9





1 LazWell Plate

LDTD-MS/MS

2 Seconds Per Well + **3 Samples Per Well** = **0.66 Seconds Per Sample**

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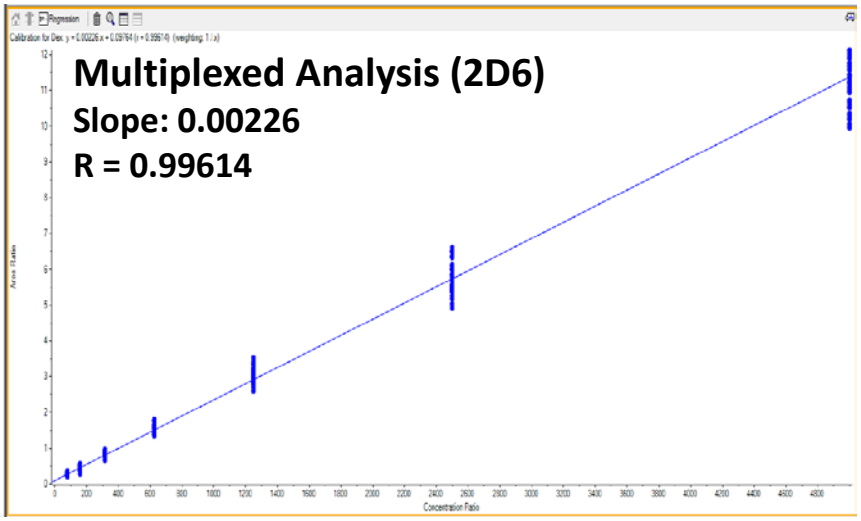
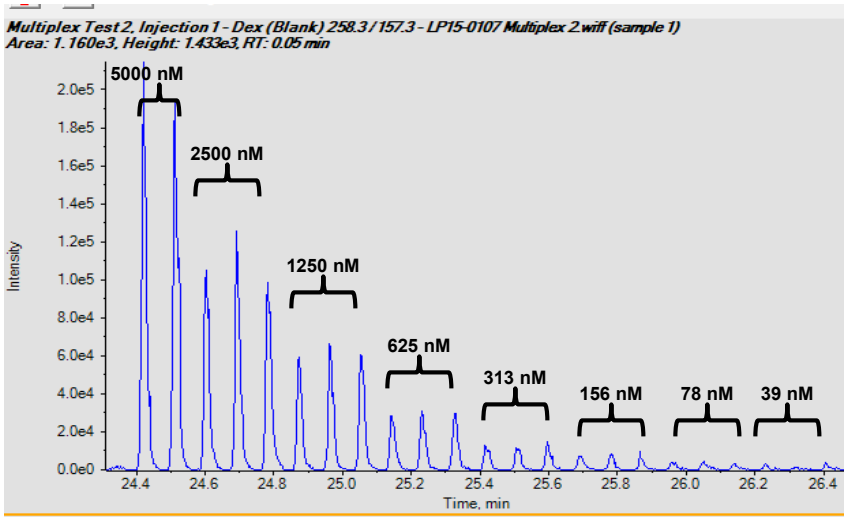
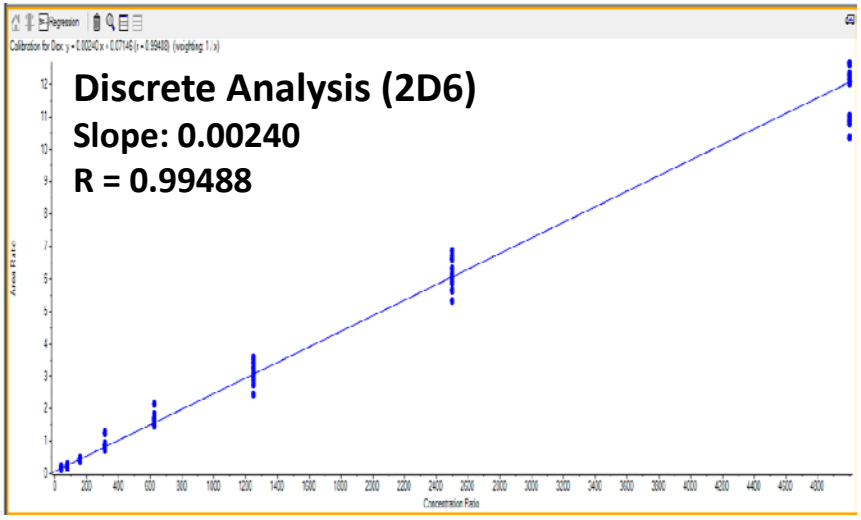
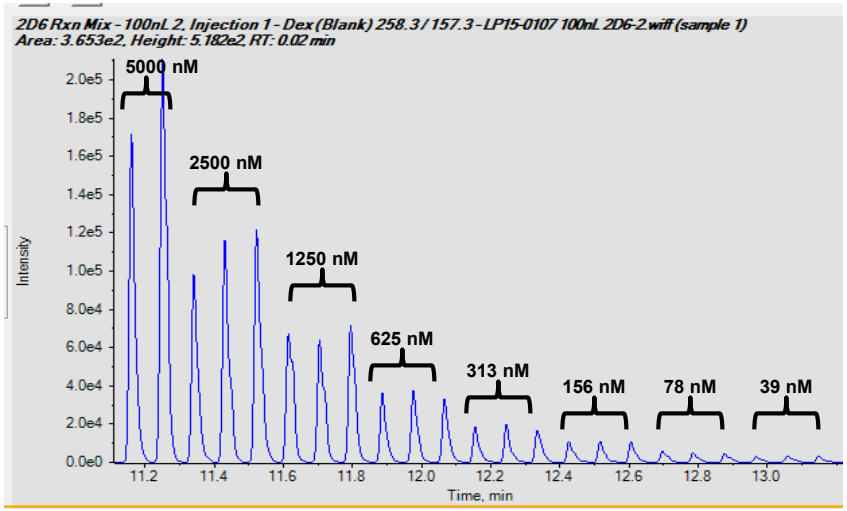
Post-Reaction Multiplexing: Data Evaluation









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HTS Evaluation – Biological Activity Screen

Goal:

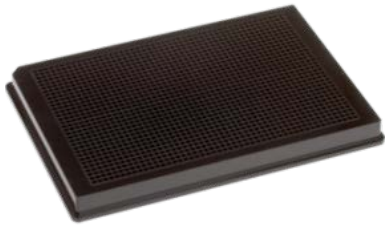
- **Develop nanoliter transfer (Mosquito HTS) with LDTD-MS/MS analysis methodology for HTS assay used to assess biological activity against a potential therapeutic target and complete a "focused" deck of representative compounds (~50,000 compounds total)**

Demonstrate:

- **Ability to successfully automate nanoliter sample transfer from 1536-well assay plates into 4 separate 384-well LazWell plates**
- **Feasibility of using LDTD to support biological activity screen**
  - **Complete entire "focused" deck screen of ~50,000 compounds**
    - **Adequate performance: Z' values, signal/background**
    - **"Hit" % comparable to fluorescence-based screen**

Desired Workflow: Nanoliter Transfer to LDTD Plates

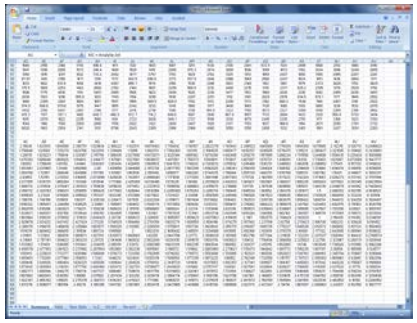
Dilute and Transfer Samples from 1536-well assay plate into 4 384-well LazWell (LDTD) plates using Mosquito HTS liquid handler (TTP LabTech)



1. Sample incubation in usual 1536-w plate

2. Dilute and Transfer nL volumes onto 4 stainless-steel LazWell 384 plate

3. LDTD-MS/MS (Phytronix) analysis



4. Peak integration and de-convolution of data

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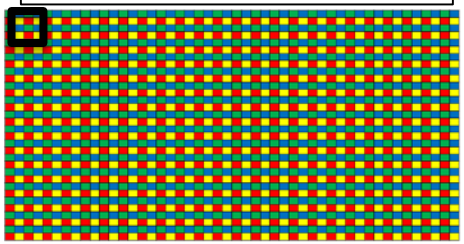
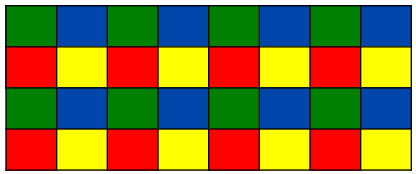
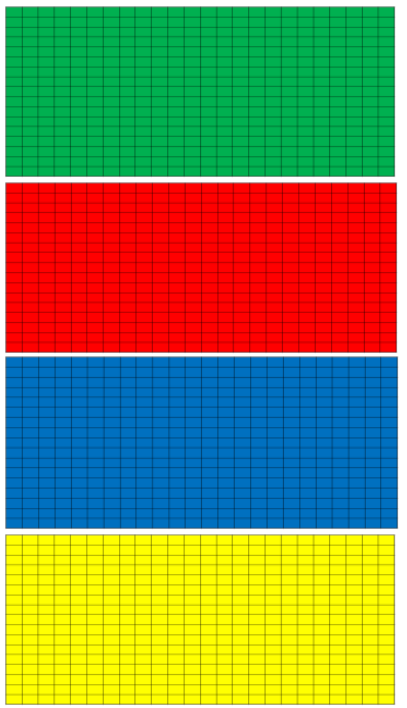
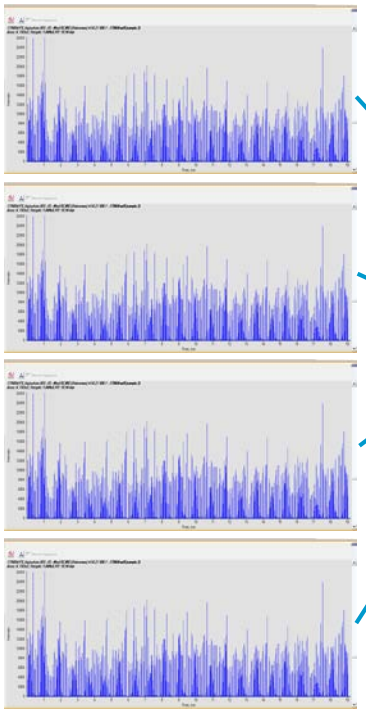
Data Processing: 1536 to 384 Workflow

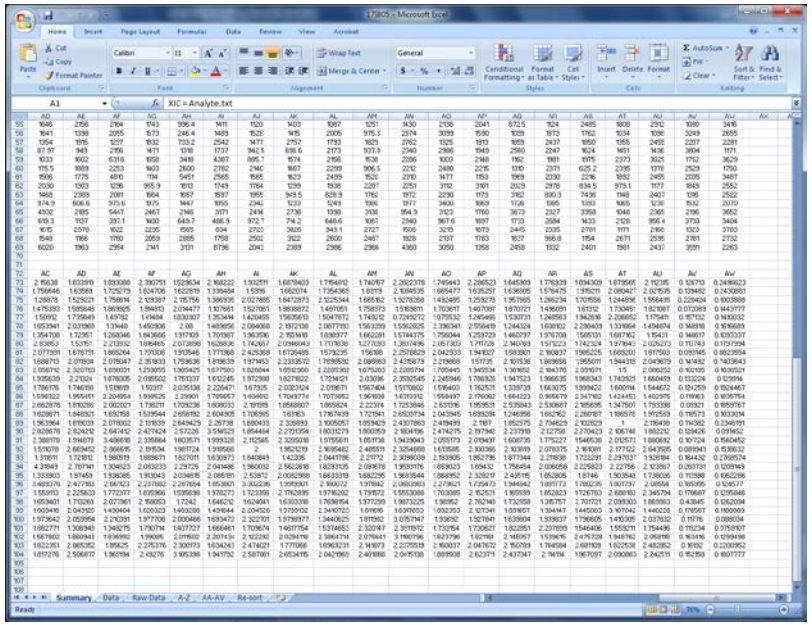
1536 Reaction Plate











384 LDTD Plates

4 Separate Data Sets

Consolidated 1536 Data Set

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Assay Validation: Concentration Response Curves (CRC) of Selected Compounds

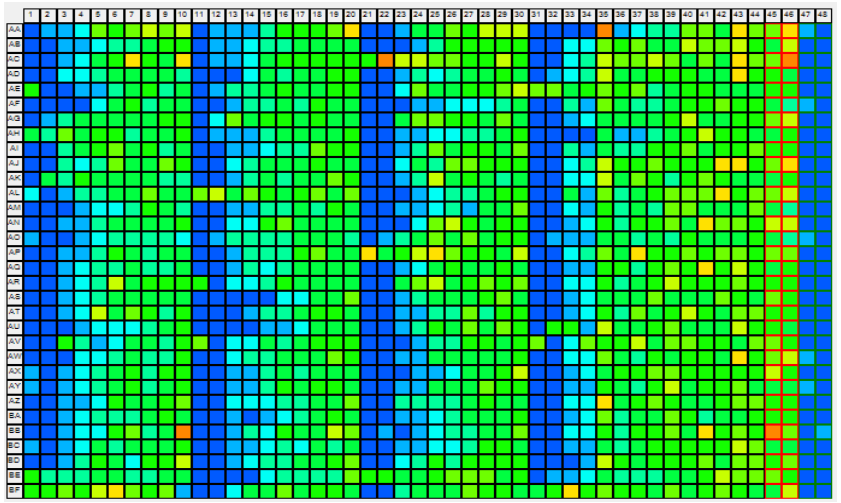
CRC Plate: Reaction

= Compounds + Enzyme + Substrate/Rxn

= Enzyme + Substrate/Rxn (+ control)

= Substrate/Rxn (- control)

126 Compounds (10 Serial Dilutions each) controls



CRC Plate: Heat Map

- Measures enzymatic activity by product:internal standard ratio

= Enzyme activity inhibited

= High enzymatic activity

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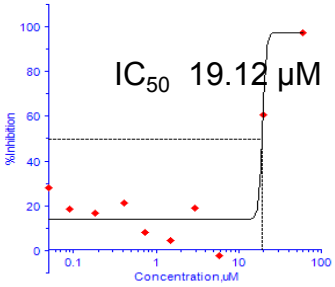
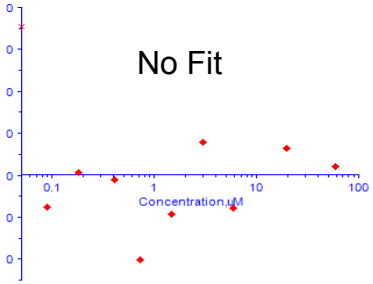
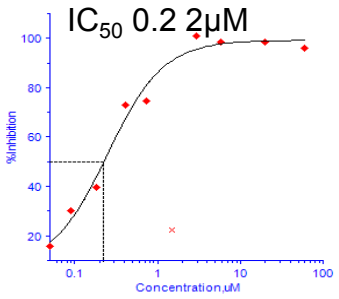
Assay Validation: CRC Results of 126 Compounds

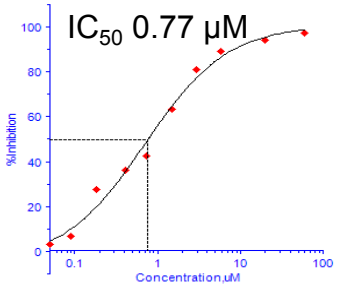
Potency Summary

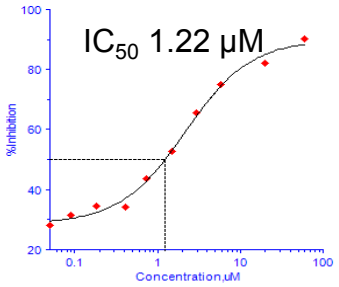
IC_{50}	Compounds
< 1 µM	23
1 – 5 µM	81
5 – 10 µM	7
>10 µM	2
No Fit/No IC_{50}	13

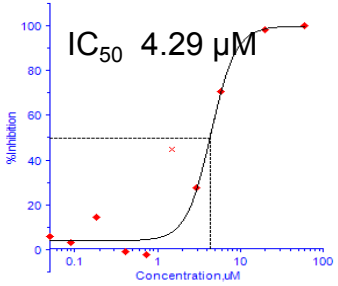


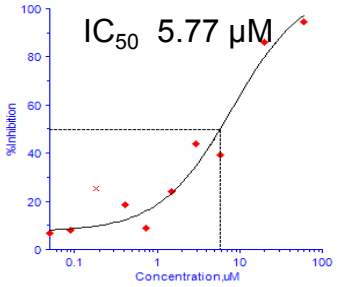


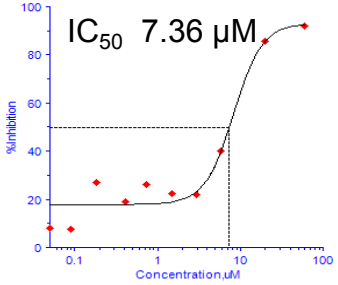












Results agree well with fluorescence assay

Completion of "Focused" Deck HTS

Primary Screen: Reaction Plate

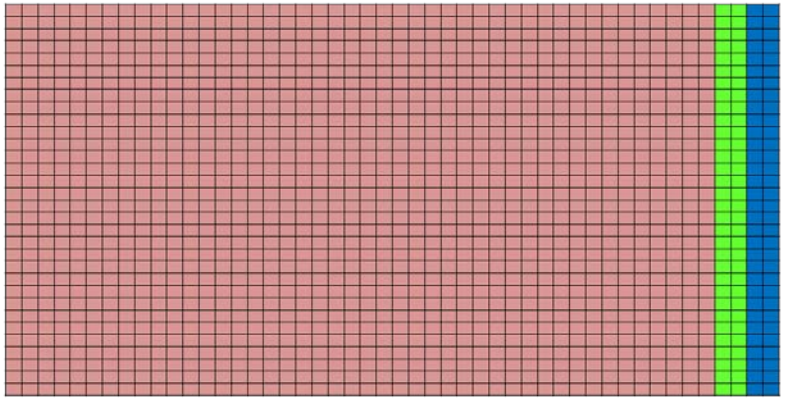
= Compounds + Enzyme + Substrate/Rxn

= Enzyme + Substrate/Rxn (+ control)

= Substrate/Rxn (- control)

1408 compounds

controls

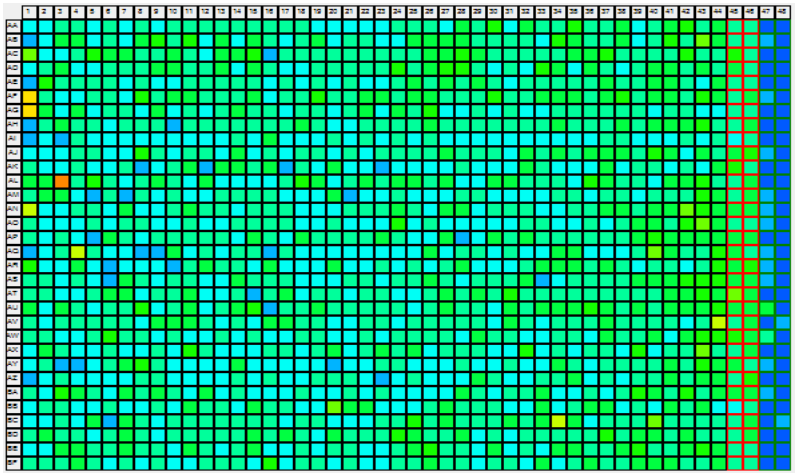


Primary Screen: Heat Map

- Measures enzymatic activity by product:internal standard ratio

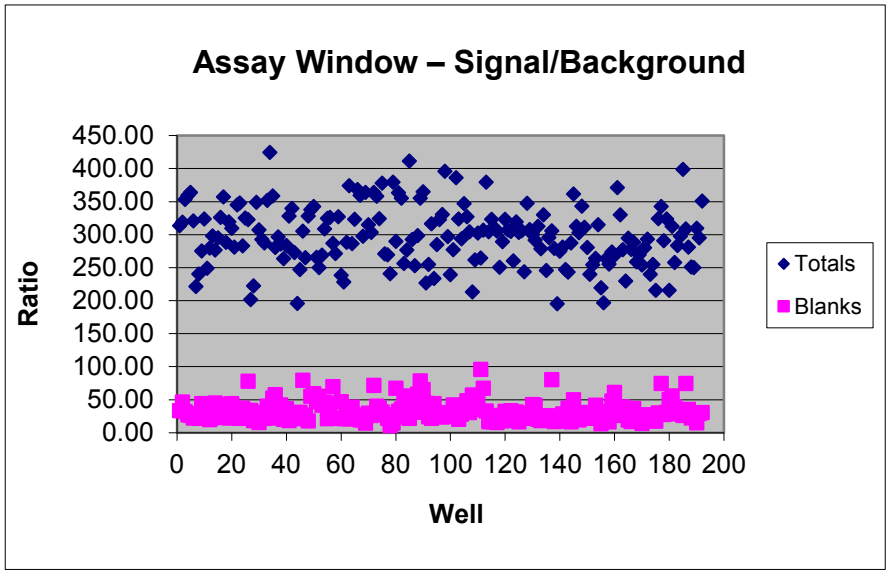
= No enzyme activity - "**Hits**"

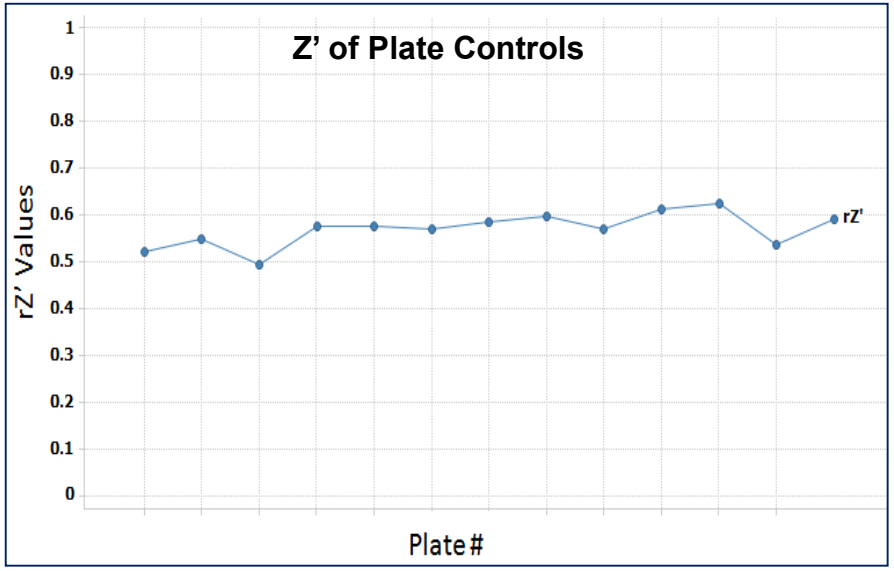
= High enzymatic activity



HTS by LDTD-MS/MS: Results and Statistics

- **HTS of ~50,000 compounds successfully/quickly completed using LDTD-MS/MS**





- Screen resulted in ~5% Hit Rate (similar to RF, Fluorescent)
- Robust signal/background
  - Totals ~10 fold higher response than blanks
- Z' of plate controls (0.56) indicate a robust screen

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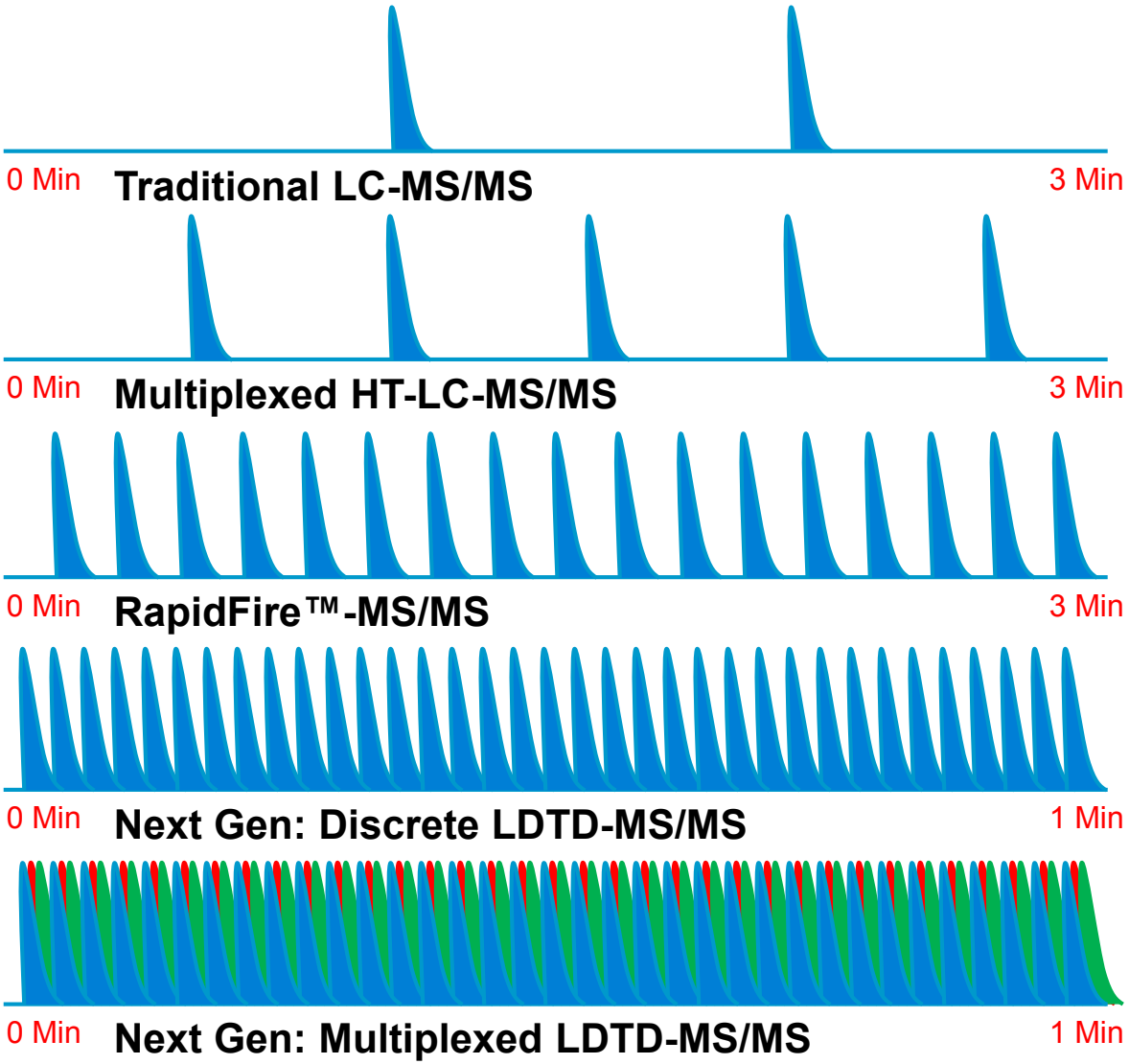
Comparison: MS-based Analytical Throughput



Many hours per 384 well plate

~3-4 hours per 384 well plate

~1 hour per 384 well plate

<15 min per 384 well plate

<5 min per 384 well plate

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Conclusions

LDTD has potential for use in both liability and activity screening applications

- Successfully completed biological activity screen
  - Quickly & successfully completed ~50,000 compound "focused" deck screen
  - Results corresponded with fluorescence and RapidFire analysis
- Completed evaluation of HT-ADME (CYP Inhibition) using LDTD-MS/MS
  - Generated IC_{50} values consistent with RapidFire analysis
  - Showed the potential for sample multiplexing

Main advantages of using LDTD-MS/MS

- 5x faster than RF discrete analysis and 16x faster multiplexed (3 separate assays)
- Greatly reduced sample volume requirements (assay miniaturization possible)

Looking forward

- LDTD can provide a complimentary approach to existing methodologies for both HT-ADME and HTS applications
- Continue to push the limitations of MS-based throughput by exploring other potential applications of LDTD technology

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