



● Ultra High Throughput Drug Discovery Screening by MALDI-TOF Mass Spectrometry powered by Simultaneous 1536 well Pipetting

Here we report the development of an automated system and workflow for ultra high throughput MALDI mass spectrometry (MS) screening (uHTS). Using the CyBio® Well vario 1536 pipettor and Bruker rapifleX MALDI-TOF/TOF MS, we have generated robust and reliable data with the potential to complete a 2 million molecule screen within 10 days.

Historically HTS of enzyme targets has utilised fluorescence formats, however these are prone to interference and need specific antibodies or tags.

Mass spectrometry offers a label free, direct detection method

of native analytes, avoiding interference and development of probes. Traditionally MS based techniques have been slow and not suited for HTS/uHTS. Matrix Assisted Laser Desorption/Ionization MS

(MALDI-MS) provides an alternative for high-throughput direct detection, with analysis times ~1 second/sample and nanoliter analyte volumes.



analytikjena
An Endress+Hauser Company

We recently described small molecule and peptide assays coupled to nanoliter dispensing, using the UltrafleXtreme MALDI-TOF for analysis (Haslam et al, 2015). However, with ~60 mins analysis read time/1536 plate, resulting in ~3 months to complete a 2 million molecule screen, throughput is still considered slow for HTS/uHTS. Subsequently we have been exploring the next generation MALDI-TOF MS and 1536 dispenser to increase throughput.

Here we describe the development of an acetylcholinesterase inhibition assay using the CyBio® Well vario 1536 pipettor and Bruker rapifleX MALDI-TOF/TOF MS. We have demonstrated analysis read times suited to uHTS (<0.25 sec/sample) with label free direct-detection offered by MALDI-TOF/TOF MS.

MALDI-MS

Analyte is co-crystallized on the surface with matrix which absorbs laser energy and facilitates desorption/ionization of analyte molecules, providing quantitation of substrate(s) and product(s) species (see figure 1).

Methodology

2.5µL of acetylcholinesterase (ACHe, 500 pM) was incubated for 10 mins before the addition of 2.5µL acetylcholine (25 µM) within 40mM Tris-HCL assay buffer. The reaction was stopped with 5 µL of 1% formic acid after 16 mins.

Sample deposition on the MALDI target (plastic and metal) resulted in a "sandwich" of matrix/analyte(s)/matrix with 100nL spot density.

A Bruker rapifleX MALDI-TOF/TOF was used in positive MS mode. Mass spectra were acquired in the range of m/z 80-1400 with 200 laser shots per sample spot.

Peak area (Acetylcholine (m/z 146.1) / Choline m/z (104.1)) were processed using MALDI PharmaPulse (MPP) software and exported for analysis into ActivityBase suite (ID Business Solutions).

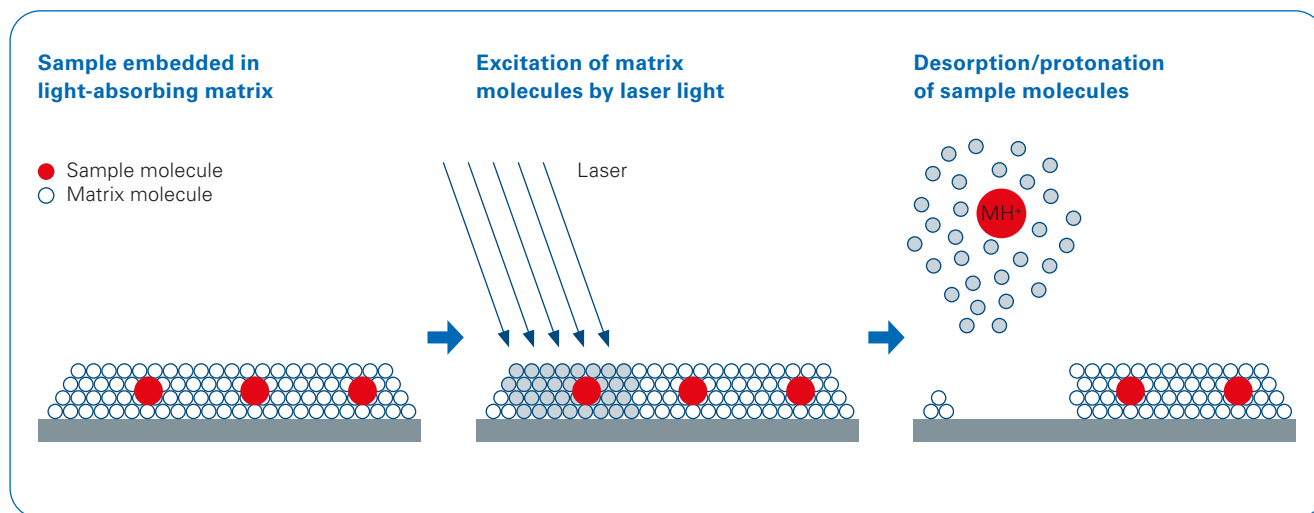


Figure 1. Schematic representation of MALDI-MS technology.

Next Generation MALDI-TOF/TOF – Bruker rapifleX

With improved spatial resolution and new laser technology the Bruker rapifleX MALDI-TOF/TOF MS enables a 20 times increase in speed when compared to existing instruments, coupled with increased robustness.

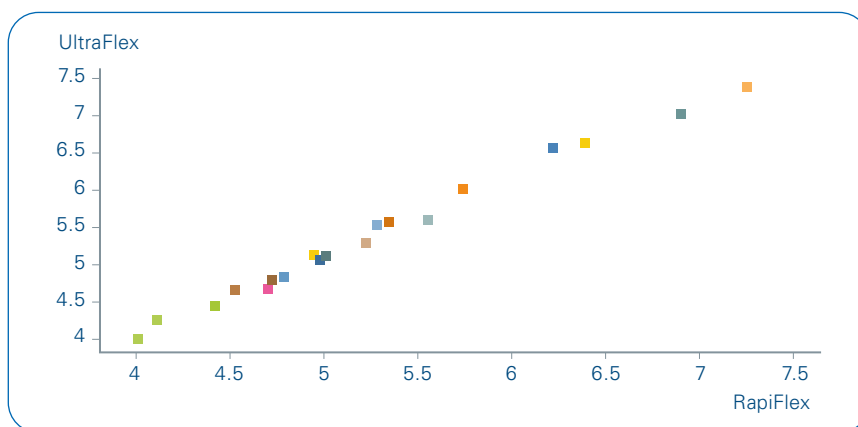
To validate this analysis read time and robustness were assessed in both single concentration and concentration response format.

Analysis read time and assay quality were significantly increased, with comparable pharmacology to ultrafleXtreme. This data demonstrated that a 2 million molecule (current GSK HTS collection) diversity screen could be completed within 10 days.

A



B



C

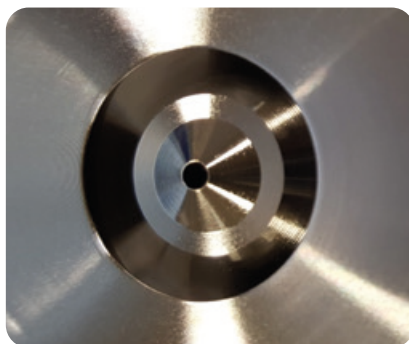
Instrument	Mean Z'
ultrafleXtreme	0.61
rapifleX	0.83

D

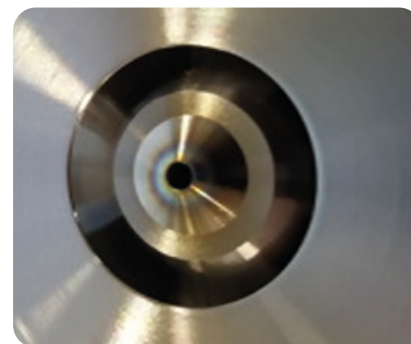
	ultrafleXtreme	rapifleX
Analysis Read Time (mins/1536 well plate)	60*	7.36
Projected Time to complete 2 million molecule screen	63	<10 days**

E

Image of Lens Stack – Before and After 2 million samples



Before



After

Figure 2. **A** Bruker rapifleX MALDI-TOF/TOF MS. **B** AChE inhibitor pIC50 values. **C** AChE inhibitor assay quality parameters. **D** Analysis read times. Analysis read times included the movement of plates into and out of the MS and concurrent cycling of the vacuum. (*) includes 20 min source clean, (**) dependant upon drying time. **E** Image of rapifleX lens stack. To analyse lens stack robustness compounds were assessed after analysis of 1536 samples (response 1) and 2 million samples (response 2), reporting no visual sample deposition, suggesting that a 2 million compound screen could be completed without a source clean.

Next Generation nanoliter pipettor – CyBio® Well vario 1536 pipettor

Having established the rapifleX was suitable for uHTS, Analytik Jena developed a 1536 channel air displacement head compatible with the CyBio® Well vario platform able to reproducibly dispense at speeds comparable with rapifleX analysis read time.

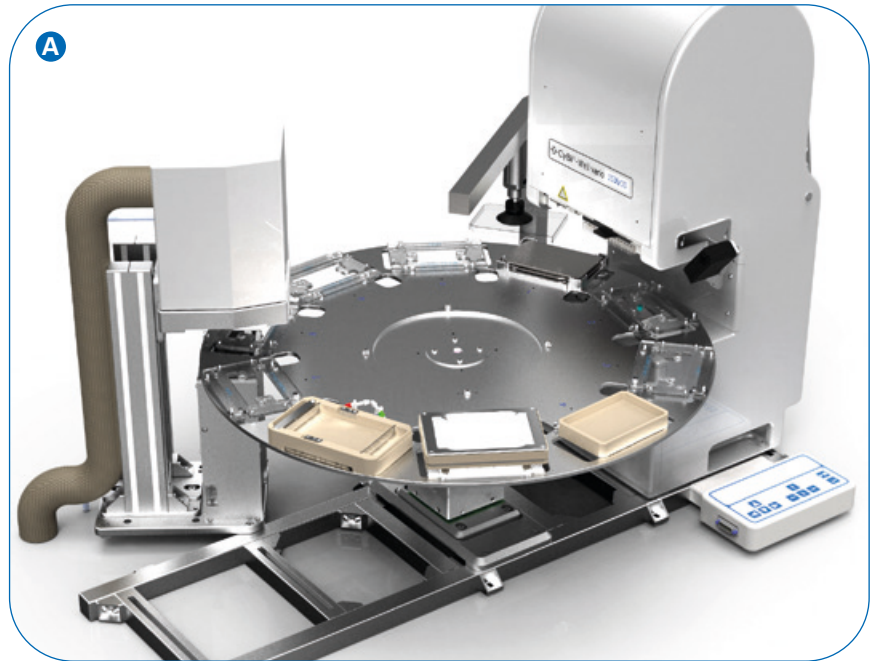
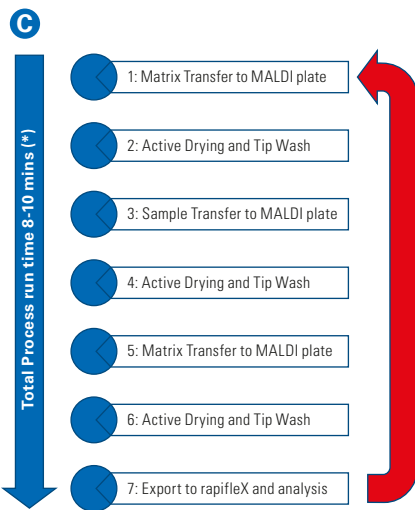


Figure 3. **A** CyBio® Well vario 1536 pipettor. **B** 1536 ceramic tips and 100nL matrix spotted on to a metal MALDI target. **C** Workflow detailing spotting/analysis process. (* dependant upon drying time).

Single Concentration Screening

Using this workflow we validated the robustness and reproducibility of spotting and analysis in single concentration format (DMSO) on both plastic and metal MALDI targets.

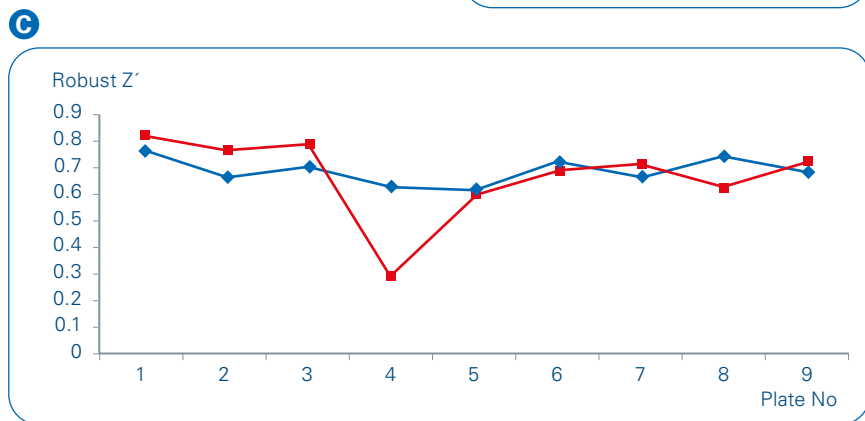
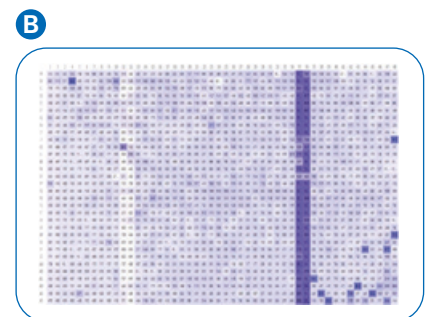


Figure 4. **A** Representative image of Cybio® Well vario spotting on metal MALDI Target. 100 nL matrix/sample/matrix was spotted using the workflow previously described. **B** Results of DMSO plate analysed within ActivityBase on metal MALDI target. Normalised % conversion ACHe inhibition data. Columns 12 and 13 uninhibited controls, 35 and 36 inhibited controls. **C** ACHe inhibitor assay Robust Z' across a nine plate run. ■ - plastic MALDI target, ◆ - metal MALDI target. Mean S:B: plastic MALDI target: 29+/-16; metal MALDI target: 13+/-2.

On Target Washing

We have developed an “On Target Wash” process to reduce or eliminate adduct formation/suppression, resulting in a increase in signal and assay robustness.

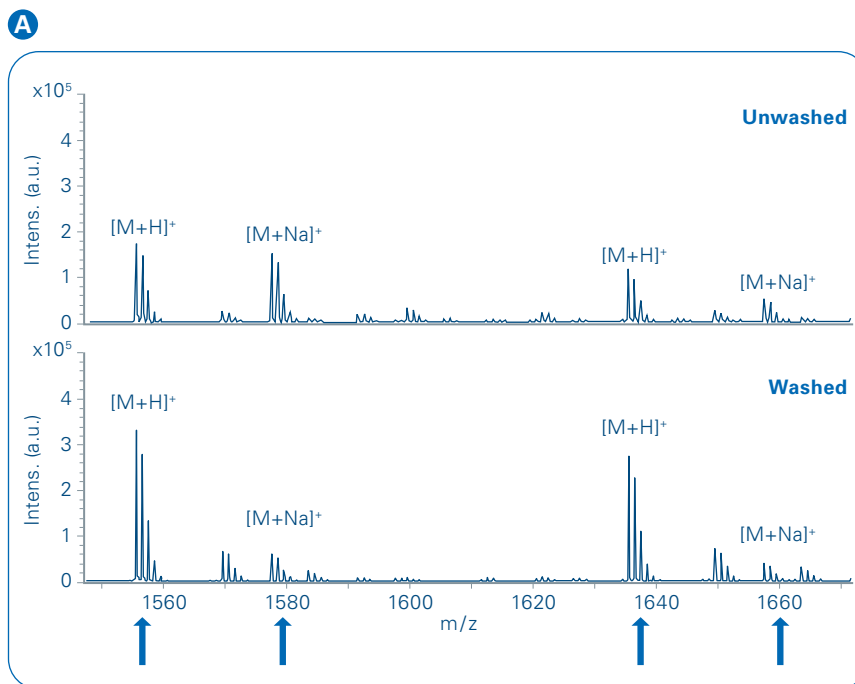
The CyBio® Well vario 1536 pipettor has been shown to complete a simultaneous 1536 wash step in less than 60 seconds (2 second dwell time) with no impact on work flow timings, whilst increasing peak area counts up to 6 fold.

Spotting and Analysis Automation System

A fully automated dispensing and analysis system was developed. Utilising this flexible platform, 1536 parallel transfer of matrix and biology onto MALDI target, drying, and insertion/removal from the rapifleX, can be completed within 7.4 mins/1536 density.

Effective scheduling ensures that up to 180 plates can be spotted and read on a daily basis.

Automated monitoring of control parameters (i.e. internal standard) allows realtime analysis to assess assay quality.



	Mean Substrate Peak Area	Mean Product Peak Area	Robust Z'
Unwashed	1016.5	649.3	0.6
Washed	6378.6	4087.9	0.9

Figure 5. **A** Mass Spectra of Washed and Unwashed analytes peak area. Using a Kinase as a model enzyme system substrate (m/z 1555) and product (m/z 1635) were analysed before and after a 300nl wash (10mM Ammonium phosphate monobasic) with a dwell time of 2 secs. **B** Peak area values and robust Z' before and after “on target washing”.

Conclusions

- The increased speed and resolution of the Bruker rapifleX MALDI-TOF/TOF instrument demonstrated highly comparable values when compared to the ultrafleXtreme, but with analysis speeds more suited to uHTS.
- When coupled to the CyBio® Well vario 1536 pipettor within an automated workflow, this process has the potential to complete a 2 million molecule diversity screen within 10 days, with fully automated processing and quality control monitoring. Additionally the “on target wash” step has the potential to increase assay robustness, reduce false positive/negative rate and minimise reagent consumption
- This technology and workflow has the potential to significantly increase productivity of early drug discovery and development.



Learn More

You are looking for further Information? Check out the link or scan the QR code for our latest webinar.

www.bruker.com/mpp-webinar



References

(Haslam et al. The Evolution of MALDI-TOF Mass Spectrometry toward Ultra-High-Throughput Screening: 1536-Well Format and Beyond, J Biomol Screen, February 2016; vol. 21, 2: pp. 176-186).

For research use only. Not for use in diagnostic procedures.

● **Bruker Daltonics GmbH & Co. KG** **Bruker Scientific LLC**

Bremen · Germany
Phone +49 (0)421-2205-0

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com – www.bruker.com