

Design of a non-LC based Affinity Selection MS breadboard system targeting 100,000 compounds screened/day

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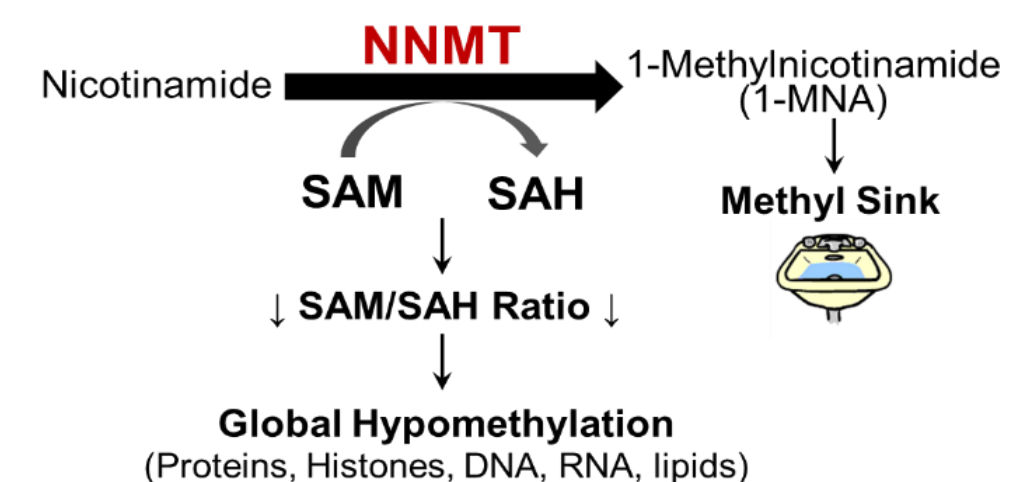
ABSTRACT

Affinity Selection Mass Spectrometry (AS-MS) is becoming an important tool in the early drug discovery process used to compliment activity based HTS screens. The concept of immobilizing the biological targets, such as enzymes or receptors, to paramagnetic beads to bypass the HPLC separation stages of bound vs. unbound library compounds was first demonstrated by Van Breemen¹. The magnetic bead approach described here uses a ferromagnetic bead-based system, an Open Port Interface, a solid-state pneumatic fluid pump and liquid surface sensing capability to increase both throughput and automation compatibility. The goal is to sustain a throughput of 100,000 compounds per day using 100 test compounds per well in 96-well plates and analyzing each well in one minute. Adding more or fewer compounds per reaction well will increase or decrease the throughput proportionally.

BREADBOARD COMPONENTS: Functions & Speeds

The system is in an initial breadboard stage prior to software integration. Each component is being tested, prior to software integration, for its ability to deliver the functions required to achieve the targeted throughputs.

- 1) Ferromagnetic beads.** Specialized 100 nM diameter high response ferromagnetic beads with magnetite cores were prototyped to serve as microscopic spin bars in order to increase reaction rates. The carboxylate surface is coated with streptavidin or nickel/cobalt to link the protein targets by conventional biotin or HisTag methodologies. When exposed to a 300 Hz rotating magnetic field each bead is distributed an equal distance from its nearest neighbor and rotates at the applied frequency. The net effect is to increase first order reaction rate 10-fold by reducing the time for the test compound to diffuse to the target compared to conventional mixers. Affinity reaction time ≈ 10 sec per sample.
- 2) Electromagnetic Mixing Apparatus (EMMA).** This agitation technology is unique in the field of magnetic beads and increased reaction rates > 10-fold as mentioned above. The 300 Hz rotating magnetic field surrounding each well in a 96 well plate is created with 4 electromagnets surrounding each well actuated in sequence with a sine wave generator. To manage heat generation 117 magnets power all 96 wells (not 4x96=384) because adjacent rows share magnets. Alternate rows spin fluid in opposite directions.
- 3) Open Port Interface^{2,3}.** The Open Port Interface aspirates and transfers to the MS the released compounds in the methanol supernatant above the beads that are fixed to the bottom of the well with a DC voltage applied to the electromagnets. It is self cleaning so avoids carry-over. Transfer time to MS ≈ 5 sec with a transport flow of 400 μ L/min methanol.
- 4) Air Infinity (AI) pump.** This unique fluid delivery system is based on microchip pressure transducers and gas pressure amplifiers. It provides the OPI with a continuous pulse free solvent flow (methanol) that delivers the sample to a conventional ESI source. The fluid is pumped by gas pressure generated from a piezo driven blowers that pressurize ambient air. OPI aspiration time ≈ 10 sec per sample
- 5) 6-Axis robot.** The sample plate is moved from station to station with a commercial miniature 6-axis robot, the Meca500, having 5-micron accuracy and precision. It serves 2 purposes. 1) It replaces conventional X-Y-Z stages for positioning the well plate for sampling by the OPI. 2) It transports the plate from station-to- station. Robot overhead is estimated to be **15 sec** per sample in fully integrated system
- 6) Multichannel pipettor.** Integra Assist Plus⁸. The target protein is covalently linked to the beads. After the binding reaction in EMMA the supernatant containing unbound compounds is discarded and methanol added to release the bound compounds. These liquid transfers are done in parallel with this small, commercially available benchtop multichannel pipettor. Liquid dispensing overhead functions estimated to account for ~ 20 s per sample but not validated yet in an integrated system.
- 7) Identification & quantification of compounds binding to target.** A high-resolution quadrupole TOF (ZenoTOF 7600 system, SCIEX) is used in the TOF only mode. For cases where there are isobaric compounds in the library, differential ion mobility (SelexION device prototype on 7600) with modifiers is used to separate the species based on their different chemical clustering properties.
- 8) Affinity Selection test assay.** Nicotinamide N-methyltransferase (NNMT)

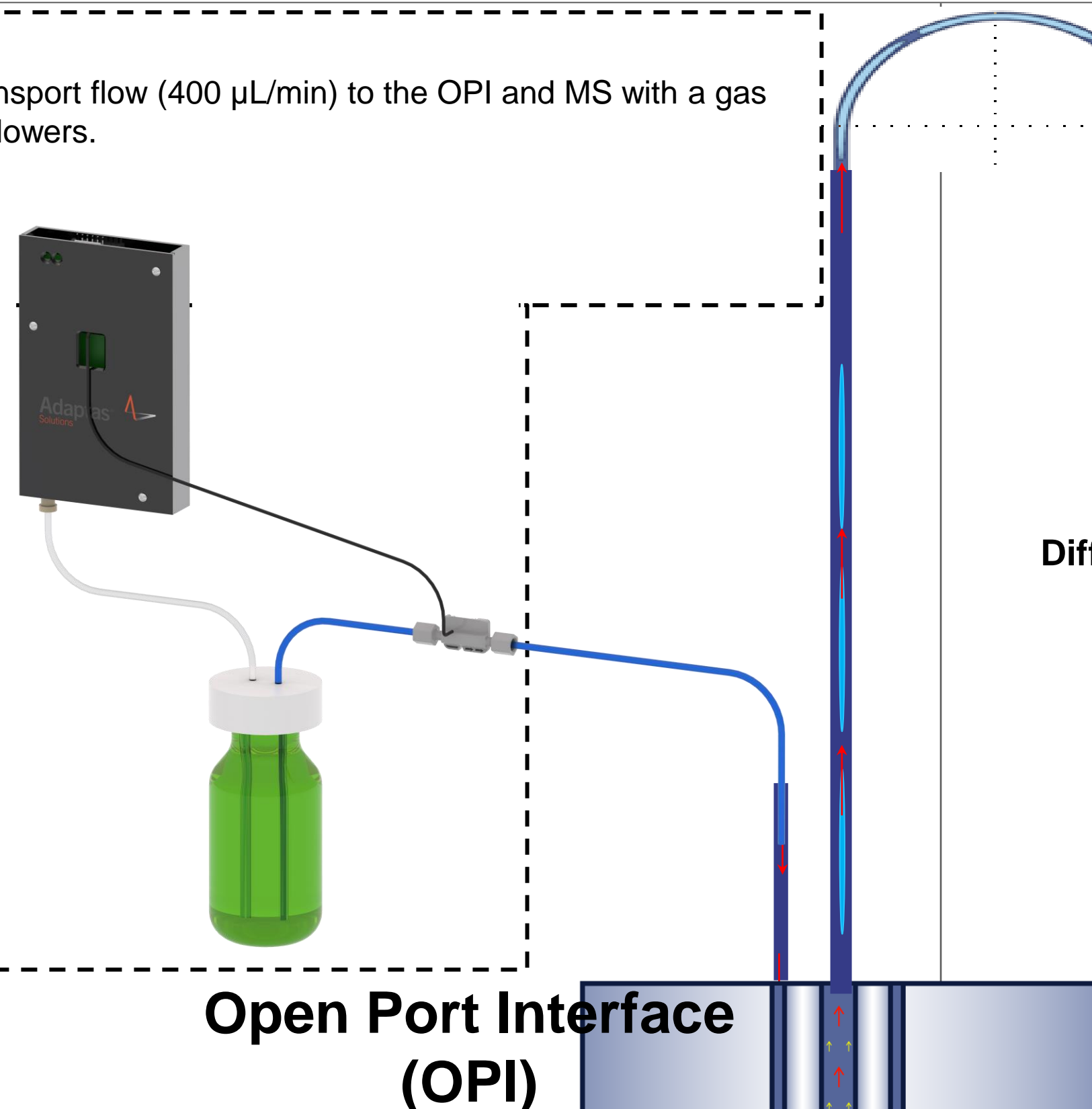


- Confirmed small molecule binding using biophysical analysis (SPR)
- Tight binders:** cmpd '960 and '399
- Weak binders:** cmpd '864 (lit cmpd) & cmpd '826 (Sanofi lit cmpd), cmpd '961 (enantiomer of '960)

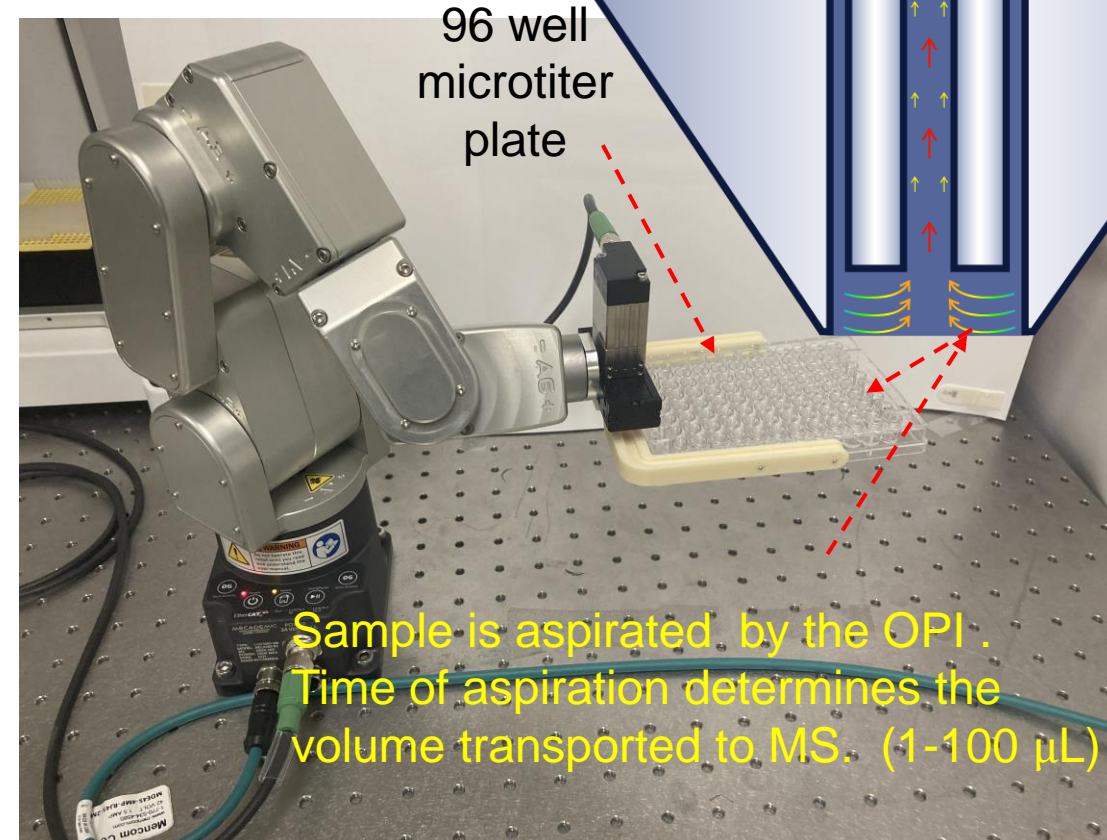
“Air Infinity”⁶ pump

Pump delivers pulse free liquid transport flow (400 μ L/min) to the OPI and MS with a gas pressure created from microchip blowers.

Microchip gas pressure amplifier



Open Port Interface (OPI)

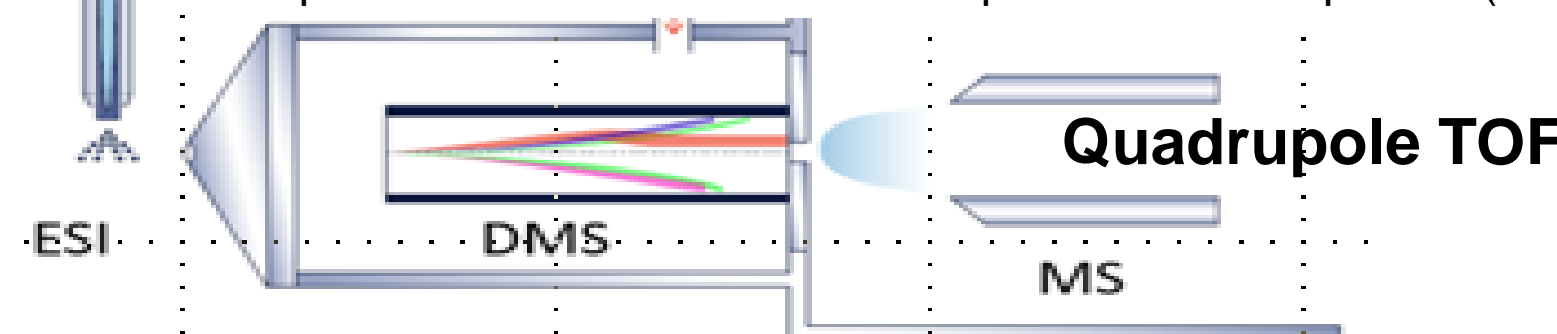


Dual purpose 6-axis robot. Meca500⁷

For X-Y-Z translation to position wells into the OPI and moves the plates from hotel to EMMA for reaction and to OPI for sampling.

Pneumatic ESI

Dual Purpose Venturi pump for quantitative sample aspiration and transport. Pressure drop created by nebulizer gas expansion is used to aspirate the sample in a quantitatively precise manner (1-100 μ L) by controlling the aspiration time. It also transports the sample to the ion source in the transport flow at 400 μ L/min (3-4 seconds).



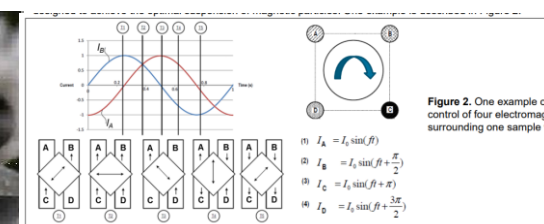
Differential ion mobility for isobar separation

300Hz Electromagnetic Mixing Apparatus (EMMA)

4 coils per well power the electromagnets



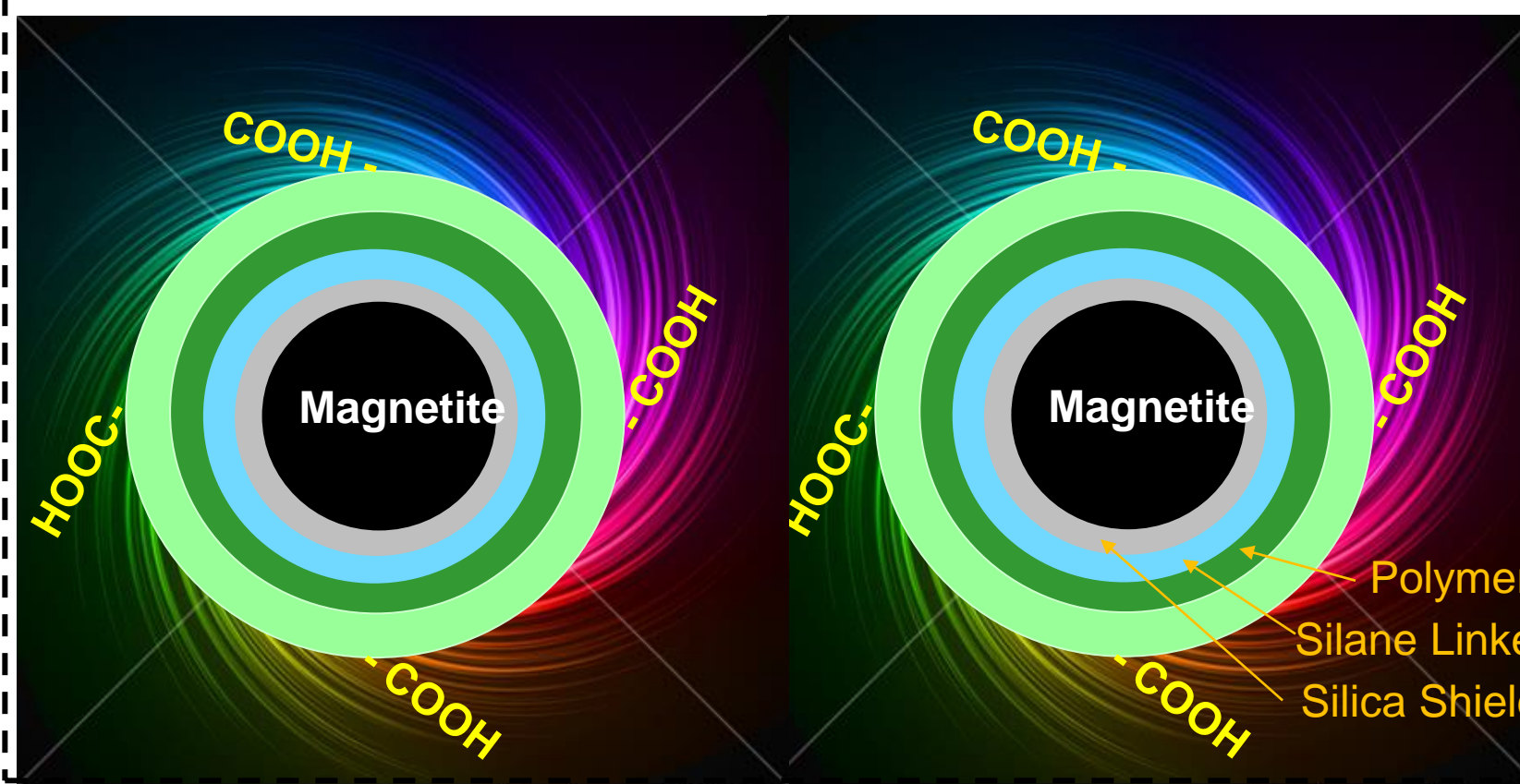
Sample wells insert into field created by star shaped electromagnets that follow the contour of the well.



Each electromagnet is powered in series by a sine wave generator to achieve 300 rotations/s

Magnetite Core Beads as High-Speed Stir Bars (F-Bars)⁴

Rotating at 300Hz they distribute 3-dimensionally equidistant throughout the sample due to the mutual magnetic self repulsion experienced at this spin frequency. Diffusion time to surface reduced > 10-fold, reaction rates increased > 10-fold. Surface of beads linked to streptavidin for biotin binding and to nickel or cobalt for HisTag binding of the protein targets.



Integra Assist Plus Multichannel Pipettor⁸.

Multichannel pipettor

OPI aspirating sample

Compound libraries

EMMA

OPI

AI Pump™ & Sensor⁶

96 well plate being sampled by OPI by aspiration

Meca500™ Robot⁷

96 well plate

AI Pump™ & Sensor⁶

96 well plate

AI Pump™ & Sensor⁶

AI Pump™ & Sensor⁶

AI Pump™ & Sensor⁶

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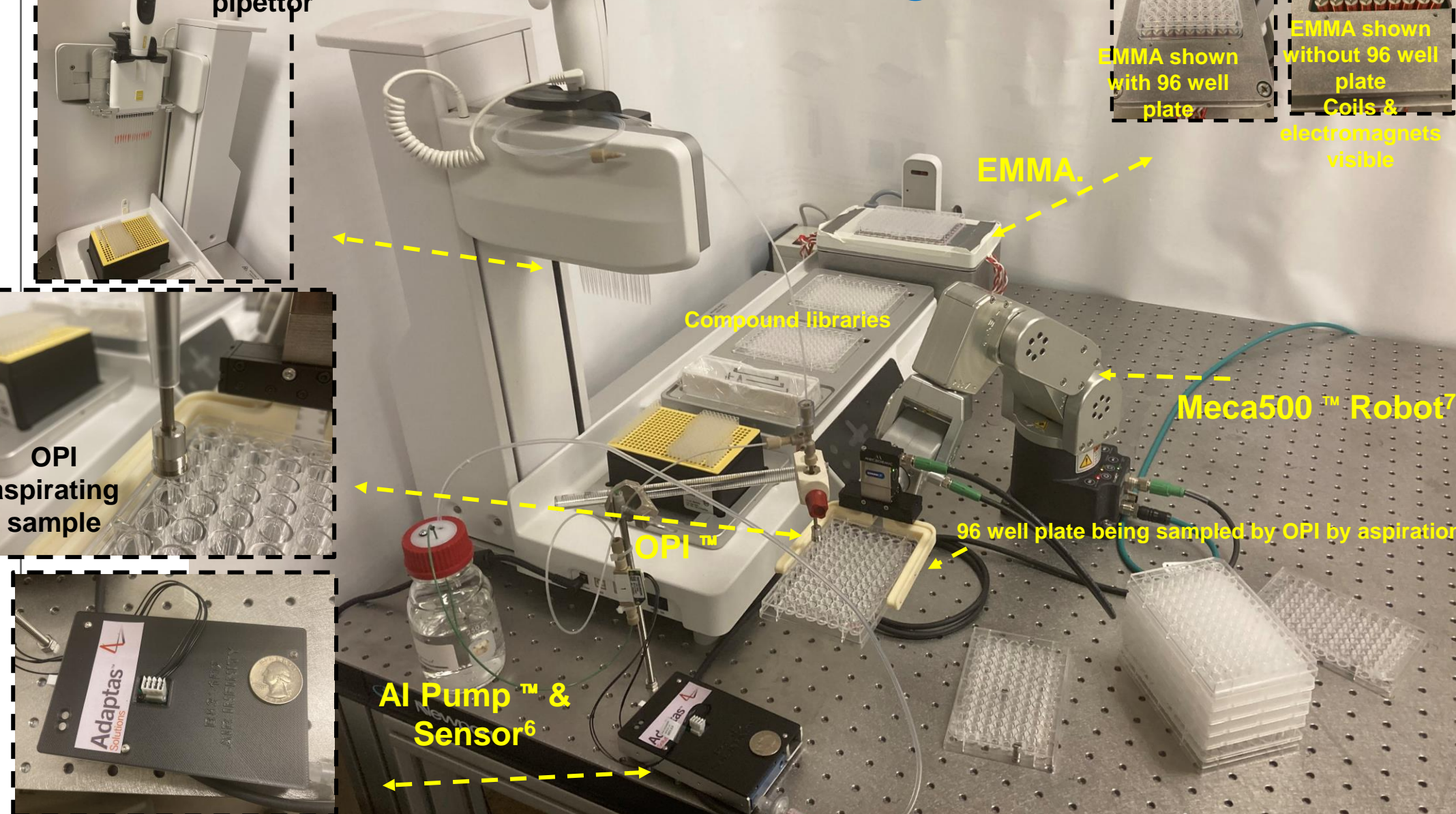
AI Pump™ & Sensor⁶

AI Pump™ & Sensor⁶

AI Pump™ & Sensor⁶

AI Pump™ & Sensor⁶

PHOTOGRAPH OF BREADBOARD



RESULTS AND CONCLUSIONS

All components of the system have been either developed in house (EMMA, ferromagnetic beads plus coatings, OPI/ORNL, differential ion mobility, prototype ZenoTOF 7600 system), prototyped by and tested with other entities (Air Infinity –Adaptas)), or purchased as unmodified commercial products (Meca500, Integra). All of these individual components have been tested individually and demonstrated they can achieve the functions and speeds required for an integrated system to process each well in < 1 minute and attain a throughput of 100,000 compounds/day screening 100 compounds in each well. The system has not yet been integrated with appropriate software communications to demonstrate that collectively these throughputs can be reached in an unattended automated system. Integration and testing is the next step in this project.

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- Integra Assist Plus, Integra Biosciences Corp, Hudson, N.H., info@IntegraBiosciences.com www.mecademic.cinfo@om/en/meca500-robot-arm

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