Analytical Methods

Supplementary Information

Title: High-Throughput screening of organic reactions in microdroplets using desorption electrospray ionization mass spectrometry (DESI-MS): Hardware and Software implementation.

Authors: Tiago Jose P. Sobreira, Larisa Avramova, Botond Szilagyi, David L. Logsdon, Bradley P. Loren, Zinia Jaman, Ryan T. Hilger, Richard S. Hosler, Christina R. Ferreira, Andy Koswara, David H. Thompson, R. Graham Cooks, and Zoltan K. Nagy.

Supplementary Tables: Table S.1

Table S.1: Item list and descriptions of the deck estate on the Biomek i7.

Position	ITEM	PART #	QTY
Orbital1	Orbital Shaker Automated Labware Positioner (ALP)	379448	1
Pos1	Positive Positioning ALP	719856	1
USW1, USW2	96/384 Pin Tool Ultrasonic Wash Station	969155	2
TL1-19	Tip Load ALP	C02867	6
P1-7	Static ALP	B87478	6
TR1-2	Trash ALP	B87478	2

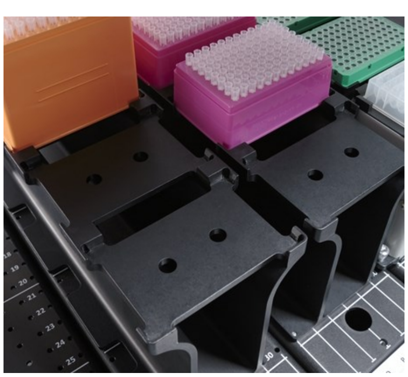
Item Description

Orbital Shaker ALP

The orbital shaker automated labware positioner (ALP) shakes the well plates and in turn mixes the reagents. Its speed is up to 1800 rpm depending on labware weight. It mounts securely to the i7 in a location accessible to the Biomek gripper and a communications cable.

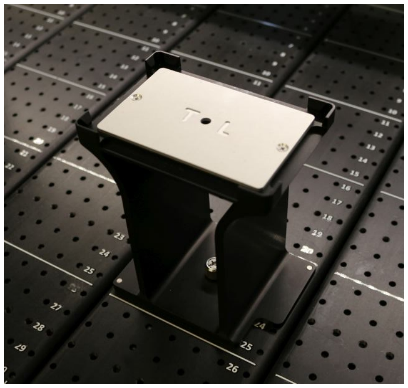
Positive Positioning ALP

The positive positioning ALP verifies the presence or absence of a microplate on the ALP and precisely positions it for interaction with the Biomek i7.



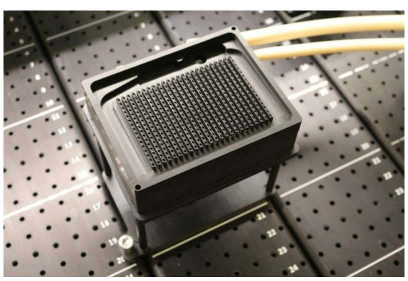
Static ALP

The Static ALP stages or stores the pipette tips and DESI substrates for use at a later time.



TipLoad ALP
The Static single position ALP serves as a stage for loading and unloading pipette tips.

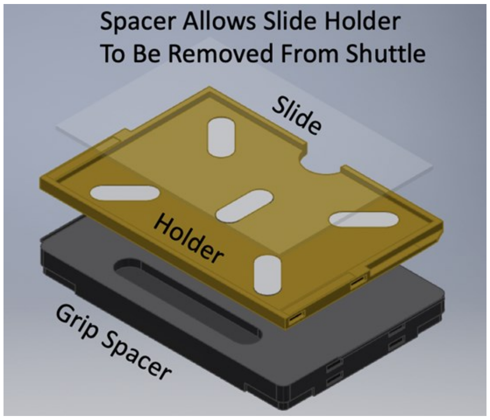
Trash ALP
The waste ALP chute is for the disposal of full or empty tip racks and microtiter plates from the work area of the Biomek.



Ultra Sonic Wash Station
The Ultra Sonic Wash Station cleans the 96/384 pin tool.

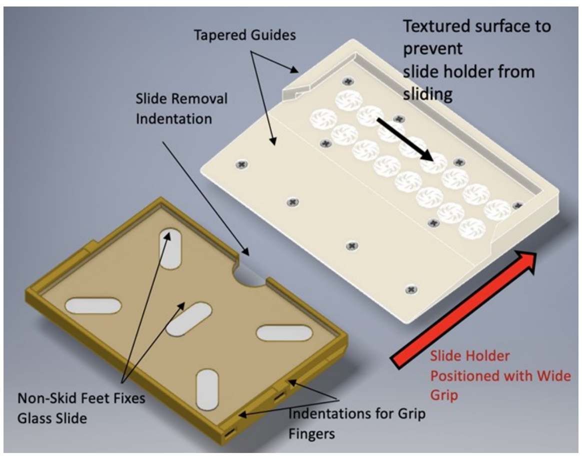
Supplementary Figures: S.1-6

Figure S.1. CAD drawing of Front (top and bottom left) and top view (bottom right) of the Biomek i7 pipetting robot with enclosure and on top of the mobile workstation.



DESI Substrate Plate Carrier

To facilitate manipulation of the glass plates on the deck of the fluid handling robot, we fabricated custom plastic plate carriers. The carriers have the footprint of a standard well plate, and the top surfaces of the plate and carrier are flush with each other to facilitate pinning.



DESI Substrate Riser plate

The plate carrier is placed on top of the riser plate for transport by the servo shuttle. Its purpose is to prevent the SCARA grip points on the plate carrier from being obstructed by the framework of the servo shuttle carriage.

Figure S.2 Design details of the DESI subsrate plate carrier and riser plate.

Figure S.3 % Breakdown of reaction outcomes accompanied with a BioMap image of a DESI substrate with 3,072 pinned reactions. The image was filtered at 443 m/z to highlight the 384 fiduciary rhodamine spots. The spots were used for visualizing the consistency of pinning by the pin tool. Ideally, all spots ahould be of equal brightness which would indicate expected ionization and consistent pinning. However, as the image shows, ion signals from ~15 spots in the yellow-highlighted area of the plate are suppressed. Additionally, reactions spotted around this area of the plate produced inconsistent product ion signal and contributes to 4% inconsistency in the total yes/no decisions.

Figure S.4 (Top) Plot of the % variance captured vs. number of PCs. 3 PCs capture ~70% of the variance and 20 PCs ~99% of the variance. (Bottom) Rhodamine spectra averaged over 3 days at 1152 replicates per plate. The plot indicates that while the variation between the specta intensity is large from sample-to-sample as indicated by the large number of PCs, the differences between the average intensity for the rhodamine samples between plates is as expected for a mass spectrum.

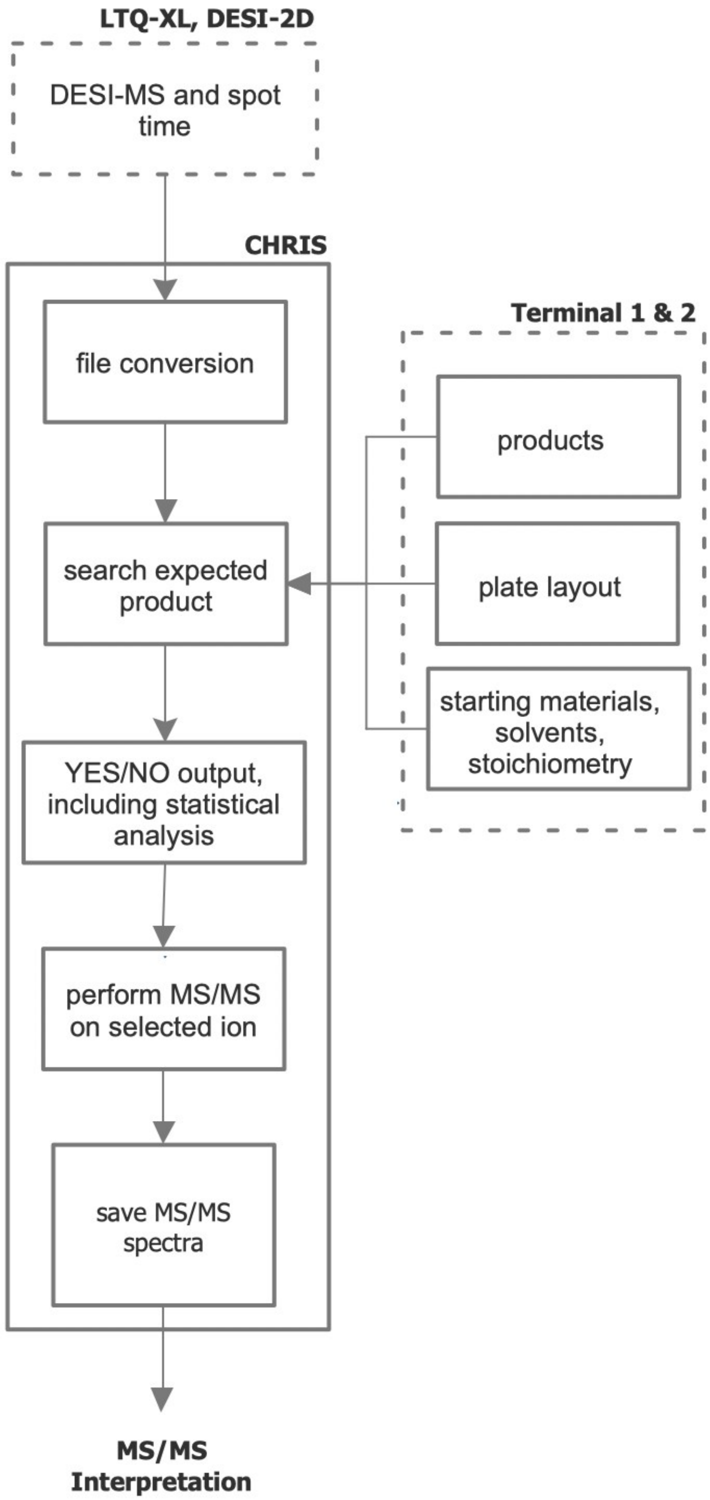


Figure S.5 Overall workflow of the DESI-MS HTS platform. Starting from user inputs from terminal 1 and 2, the input parameters are cascaded to CHRIS, which then interact and supervise with all the software and hardware. CHRIS converts the MS file format to a standard file format for proper communication across all software, searches the expected product, compiles data for statistical analysis, performs MS/MS on selected ion, and saves the spectra for further interpretation.

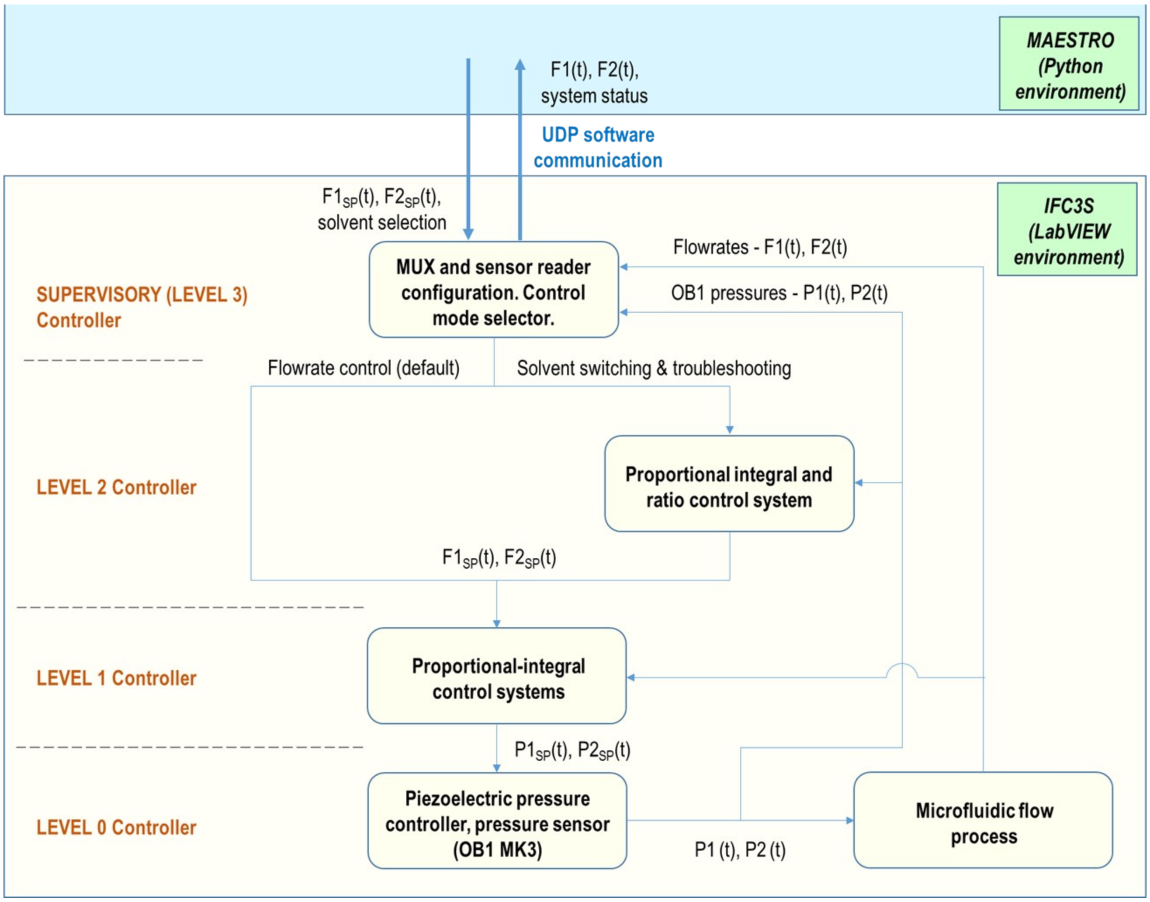


Figure S.6 The hierarchical control strategy applied within the IFC3S. A two-level problem identification and troubleshooting protocol using PI and ratio control enables detection and handling of destabilization of the DESI spray solvent flow without prolonged exposure to the DESI substrate

ACN

	6	7	8	9	10	11	12	13	14	15	16	17
1	2.84E-04	1.27E-01	1.18E-04	3.29E-04	4.11E-02	1.91E-01	6.67E-04	5.56E-06	5.06E-01	6.35E-02	2.59E-01	3.39E-01
2	1.59E-02	7.32E-02	1.19E-02	1.27E-02	1.53E-02	9.65E-02	6.26E-03	2.41E-04	9.50E-02	4.28E-03	1.14E-01	1.86E-01
3	1.81E-01	3.54E-01	1.61E-01	1.59E-01	1.09E-01	1.75E-01	1.36E-01	2.23E-02	1.55E-01	1.60E-02	1.33E-01	2.31E-01
4	4.73E-04	3.30E-03	1.08E-02	3.85E-03	9.29E-04	1.16E-02	2.48E-03	3.92E-03	2.16E-01	2.43E-02	2.01E-01	2.94E-01
5	6.01E-01	6.31E-01	5.44E-01	3.00E-01	5.17E-01	7.79E-01	3.89E-01	4.27E-02	2.18E-01	5.74E-02	1.63E-01	3.14E-01

DMSO

	6	7	8	9	10	11	12	13	14	15	16	17
1	4.71E-04	3.81E-04	6.09E-04	8.01E-04	3.31E-04	9.52E-04	3.62E-04	1.85E-06	1.48E-01	1.64E-02	1.17E-01	1.56E-01
2	3.64E-03	2.99E-03	4.92E-03	7.34E-03	1.56E-03	4.10E-03	1.11E-03	1.75E-04	5.20E-02	3.84E-03	2.52E-02	2.20E-02
3	2.64E-02	2.85E-02	5.49E-03	2.94E-02	3.39E-02	6.78E-02	2.77E-02	1.99E-03	7.03E-02	6.91E-02	7.32E-02	1.87E-01
4	2.01E-05	4.19E-03	1.28E-02	5.46E-03	7.73E-04	8.32E-04	3.23E-04	1.34E-02	8.64E-02	3.05E-02	8.80E-02	2.13E-01
5	1.92E-01	2.20E-01	6.48E-02	1.27E-01	2.53E-01	3.58E-01	1.33E-01	2.69E-02	6.34E-02	8.69E-02	8.17E-02	1.45E-01

Toluene

	6	7	8	9	10	11	12	13	14	15	16	17
1	4.35E-04	7.01E-02	2.80E-03	4.42E-04	3.37E-02	1.08E-01	2.56E-04	9.26E-06	2.70E-01	3.80E-03	1.35E-01	3.51E-01
2	4.65E-03	2.92E-02	6.76E-03	4.17E-03	3.80E-03	3.30E-02	1.23E-03	1.36E-04	3.66E-02	3.10E-03	3.59E-02	4.45E-02
3	2.36E-01	3.49E-01	1.55E-01	1.56E-01	8.78E-02	1.13E-01	9.81E-02	4.59E-03	3.13E-02	2.37E-03	3.63E-02	1.78E-01
4	9.38E-04	4.39E-03	7.66E-03	4.07E-03	1.72E-03	1.70E-02	4.40E-03	4.36E-03	9.79E-02	2.04E-03	1.57E-01	2.80E-01
5	5.42E-01	6.01E-01	4.66E-01	3.06E-01	5.01E-01	7.73E-01	3.84E-01	1.92E-02	1.24E-01	2.78E-02	1.42E-01	3.04E-01

Figure S.7. Heat maps showing the normalized intensity of the products from the reactions of the amines (**1**-**5**) and electrophiles (**6**-**17**) in three different reaction solvents (ACN, DMSO, and toluene). Each value reported is an average across all plates and all days (a total of 144 or 270 replicates depending on the electrophile). Dark red indicates the areas of highest intensity, and dark blue indicates the areas of lowest intensity.

Additional Description of Figure S.7:

Figure S.7 contains heat maps showing the results of the reactions conducted in this study. The results show that ACN is the most universal solvent in terms of promoting reactivity. ACN was the most effective solvent for reactions involving sulfonyl chlorides (**6**-**9**) and acyl chlorides (**10**-**13**) while also showing significant product formation with reactions involving alkyl bromides (**14**-**17**). DMSO proves to be the least effective solvent for reactions with sulfonyl chlorides and acyl chlorides; however, it is the best solvent for reactions with 2-(bromoethyl)-benzene (**15**). 2-(bromoethyl)-benzene reacts predominantly via the S_N2 mechanism because the leaving group is attached to an ethylene moiety. Perhaps DMSO is effective at stabilizing the pentacoordinate transition state which occurs during S_N2 reactions. Toluene, a nonpolar solvent, proves to be less effective for reactions with alkyl bromides (**14**-**17**). Alkyl bromides **14**, **16**, and **17** contain leaving groups attached to benzylic carbons, which react predominantly via the S_N1 mechanism. Toluene is expected to be a poor solvent in these cases because S_N1 reactions favor polar solvents that can stabilize the cationic intermediate that forms during these reactions. 3-(2-methylpiperidin-1-yl)propan-1-amine (**5**) was the most reactive amine used. It was the only primary amine and therefore the least sterically hindered, which explains it high reactivity. The more electron rich, methoxy substituted 4-methoxybenzenesulfonly chloride (**7**) and 4-methoxybenzoyl chloride (**11**) were the most reactive among the sulfonyl chlorides and acyl chlorides used. As mentioned above, 2-(bromoethyl)-benzene reacts predominantly via the S_N2 mechanism while the other alkyl bromides react predominantly via the S_N1 mechanism, and it appears that this mechanistic difference causes the reactions with 2-(bromoethyl)-benzene to be less favorable.

Supplementary Video: I7_CHRIS_MSMS.mp4

This video shows the capability of the DESI-MS HTS platform and CHRIS to perform automated MS and MS/MS data acquisition in real time. The time stamps for the operation are:

[0:00] Pinning of a DESI plate using a pin tool
[1:00] DESI Plate transfer from the Biomek i7 to the DESI-2D stage
[1:24] CHRIS initialization, including calibration of initial spray position.
[1:30] Automated MS data collection - the video speed is increased 20X from 1:55 - 2:11
[2:18] Demonstration of CHRIS graphical input and output interface accompanied with ThermoFisher's Tune Plus MS acquisition software and a camera pointing at the MS inlet capillary
[2:44] MS/MS output results shown in ThermoFisher's Xcalibur Qual Browser.