Supporting Information

Title: Parallelization with Dual-Trap Single-Column Configuration Maximizes Throughput of Proteomic Analysis

Authors

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No	Command	Parameters
1	UdpInjectMarker	
2	UdpInjectValve	Load
3	UdpDraw	ReagentBVial, 25 [µl], 0.25 [µl/s], GlobalHeight
4	UdpInjectValve	Inject
5	UdpDispense	Drain, 25 [µl], 1 [µl/s], GlobalHeight
6	UdpMixWait	60 [s]
7	UdpInjectValve	Load
8	UdpDraw	ReagentAVial, 25 [µl], 0.25 [µl/s], GlobalHeight
9	UdpDispense	Drain, 25 [µl], 1 [µl/s], GlobalHeight
10	UdpDraw	ReagentAVial, 25.000 [µl], 0.25 [µl/s], GlobalHeight
11	UdpDispense	Drain, 25.000 [µl], 1 [µl/s], GlobalHeight
12	UdpMixWait	180 [s]
13	UdpDraw	SampleVial, 20 [µl], GlobalSpeed, GlobalHeight
14	UdpDraw	ReagentAVial, 2.4 [µl], GlobalSpeed, GlobalHeight
15	UdpInjectValve	Inject
16	UdpDispense	Drain, 22.400 [µl], GlobalSpeed, GlobalHeight
17	UdpSyringeValve	Needle
18	UdpMixNeedleWash	50 [µl]
19	UdpMoveSyringeHome	GlobalSpeed

Table S1. Program for operation of auto-sampler (same in both methods). Reagent B vial contained 0.1% formic acid in acetonitrile. Reagent A vial contained 0.1% formic acid in water.

#MS Type	Cycle Id	Start IM [1/K0]	End IM [1/K0]	Start Mass [m/z]	End Mass [m/z]	CE [eV]
MS1	0	-	-	-	-	-
PASEF	1	1.0711	1.3447	1000	1040	-
PASEF	1	0.8342	1.0511	640	680	-
PASEF	1	0.65	0.8226	360	400	-
PASEF	2	1.0974	1.3774	1040	1080	-
PASEF	2	0.8605	1.0837	680	720	-
PASEF	2	0.6763	0.8553	400	440	-
PASEF	3	0.9132	1.1489	760	800	-
PASEF	3	0.7026	0.8879	440	480	-
PASEF	4	0.9395	1.1816	800	840	-
PASEF	4	0.7289	0.9205	480	520	-
PASEF	5	0.9658	1.2142	840	880	-
PASEF	5	0.7553	0.9532	520	560	-
PASEF	6	0.9921	1.2468	880	920	-
PASEF	6	0.7816	0.9858	560	600	-
PASEF	7	1.0184	1.2795	920	960	-
PASEF	7	0.8079	1.0184	600	640	-
PASEF	8	1.1237	1.41	1080	1120	-
PASEF	8	0.8868	1.1163	720	760	-
PASEF	9	1.0447	1.3121	960	1000	-

Table S2. DIA isolation window scheme for DIA-PASEF method optimized for the presented platform and whole blood from Mitra devices.

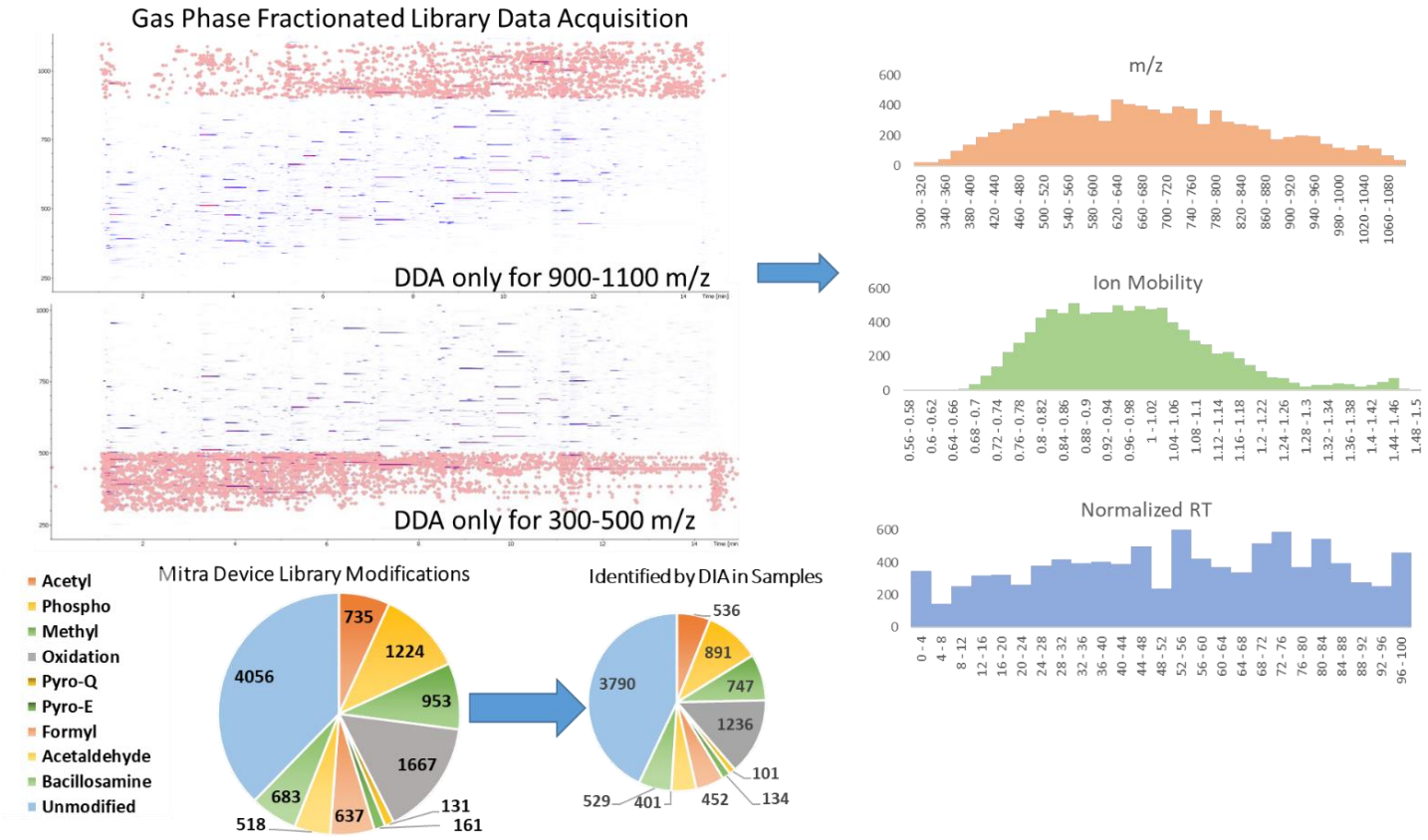
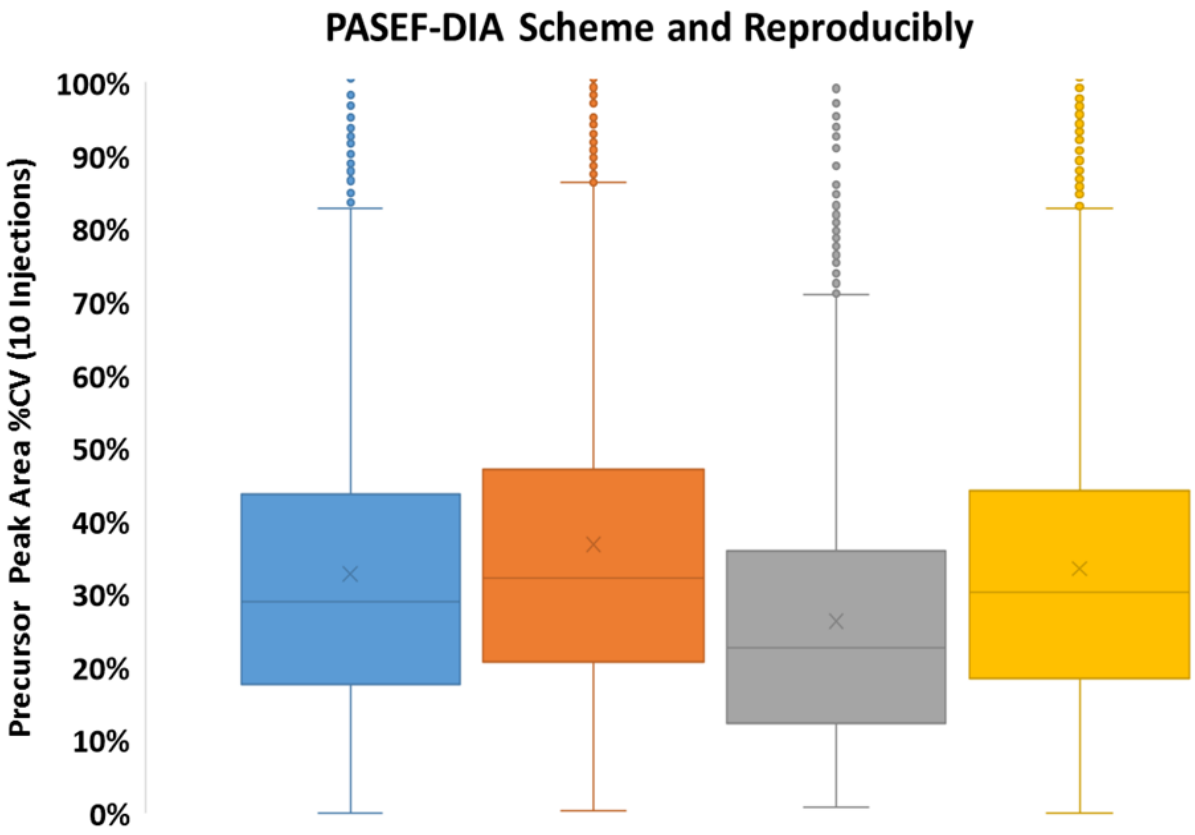


Figure S1. Gas phased fractionated data dependent acquisition was used to generate the library for analysis of blood from Mitra devices. In each run the m/z range of precursors allowed for fragmentation was limited to 200 m/z, top left are two example heat-maps that show the detected precursor ions while the pink diamonds indicate those which were fragmented. The histograms on the left represent the m/z, ion mobility, and retention time (RT) distribution of the identified precursor ions that were incorporated into the library. The pie charts present the modifications which were identified in the library (bottom left) and later identified in DIA analysis of the Mitra device samples. (bottom right)

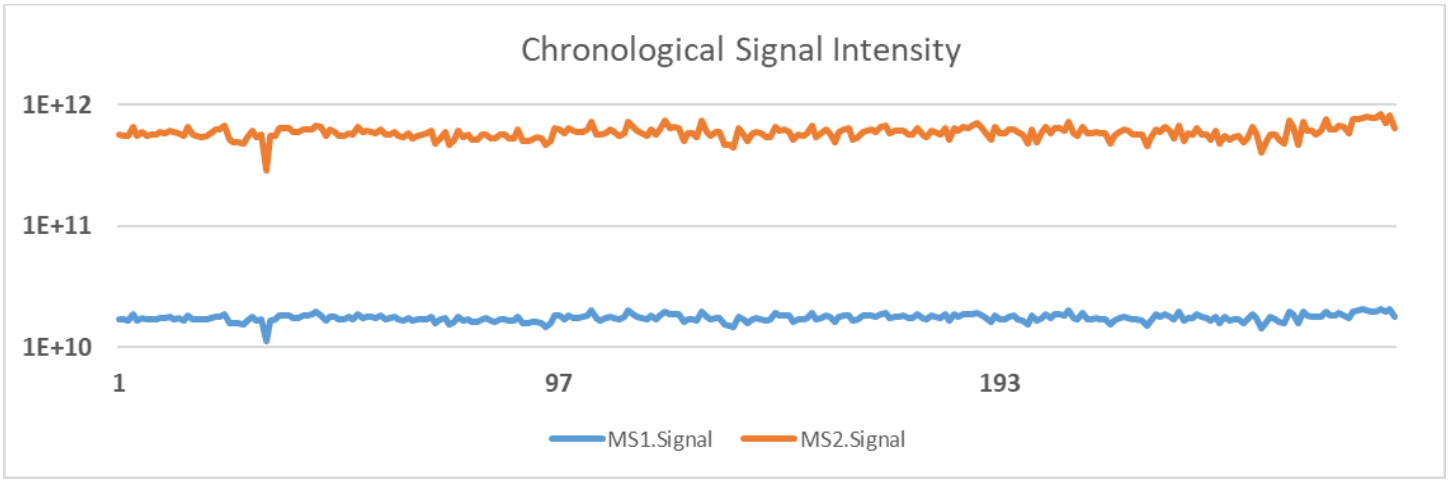


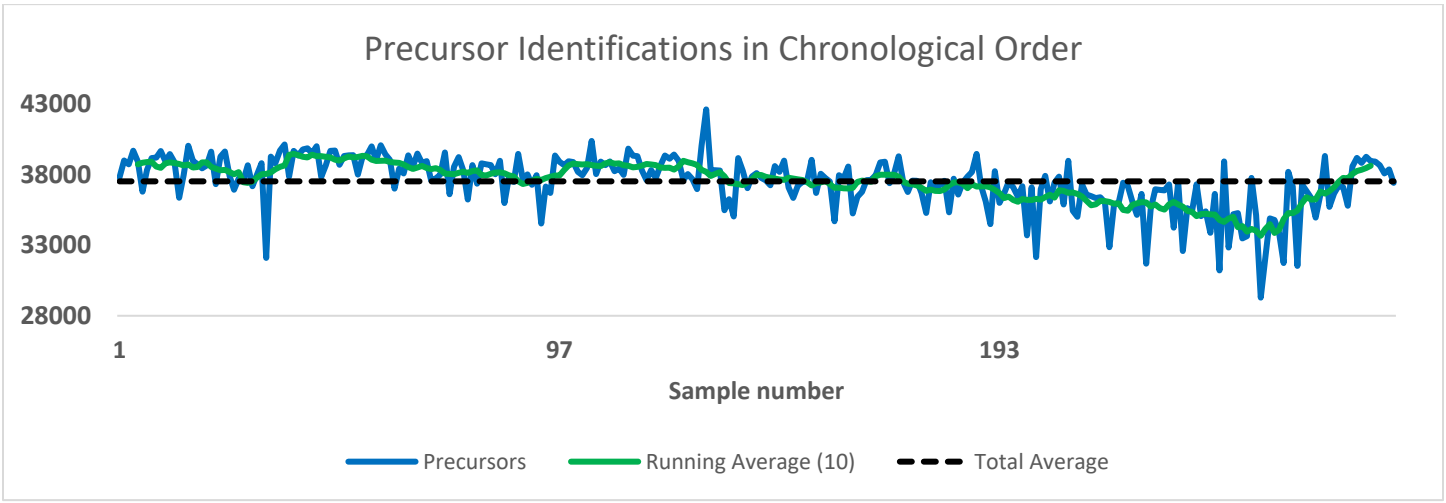
	60ms ramp 25mz IW	85ms ramp 50mz IW	70ms ramp 50mz IW	50ms ramp 30mz IW
Median CV	29%	32%	23%	30%
Average CV	33%	37%	26%	33%
Quantified Precursors	3765	3985	3985	3884
Average Precursors	3379	3616	3697	3525
MS2 Scans FWHM	2.9	2.7	3.1	2.7

Figure S2. Optimization of DIA scheme for 15 minute run time. Increasing the number of MS2 scans at across the peak (MS2 scans FWHM) reduced quantitative variance (%CV) and the best method used a 70 ms ramp with 50 m/z wide isolation windows. The final DIA method used a 70 ms ramp and 40 m/z windows but a more focused precursor m/z and mobility range which resulted at 0.76 s data acquisition cycle and 3.3 to 4.0 MS2 scans at FWHM in subsequent experiments.

	F1	AP	Recall	Specificity	Accuracy	AUROC	AUPR
Dummy	0.682927	0.518519	1.000000	0.000000	0.518519	0.500000	0.759259
ET	0.756757	0.608696	1.000000	0.307692	0.666667	0.901099	0.913790
GB	0.764706	0.640608	0.928571	0.461538	0.703704	0.774725	0.755464
LR	0.823529	0.700000	1.000000	0.538462	0.777778	0.873626	0.876529
RF	0.800000	0.666667	1.000000	0.461538	0.740741	0.807692	0.790904
SVC	0.823529	0.700000	1.000000	0.538462	0.777778	0.873626	0.876529

Table S3. Scores of different classifier models used to distinguish normal controls from hypertension patients.





	1	2	3	4	5	6	7	8	9	10	11	12
A	36.6	38.2	37.1	39.0	36.1	36.6	37.5	36.6	37.8	38.2	39.3	39.2
B	37.5	36.0	32.2	35.4	32.9	31.7	32.6	31.2	35.1	37.1	35.7	38.8
C	37.9	36.7	37.0	35.0	35.9	35.6	35.6	38.9	29.3	31.5	36.7	39.3
D	38.2	37.5	37.9	37.3	36.0	37.0	35.4	32.8	32.1	37.3	37.2	39.0
E	39.5	37.1	36.1	36.6	37.4	36.9	37.3	35.2	34.9	36.8	38.4	38.9
F	37.2	36.4	37.4	36.5	37.2	36.9	35.1	35.3	34.8	36.3	37.1	38.7
G	36.1	37.2	37.9	36.3	36.1	37.3	35.4	33.5	33.9	35.0	35.8	38.1
H	34.5	33.7	35.9	36.4	35.2	34.3	33.9	33.6	31.7	36.5	38.6	37.4

Figure S3. Platform Performance across the cell lysate analysis. Top – MS1 and MS2 signal over course of the experiment. Middle – identifications over the course of the cell lysate analysis. Bottom – precursor identifications across plate 3 which had the highest variability.