

# **Supplemental Information for**

## **Dynamic range expansion by gas-phase ion fractionation and enrichment for imaging mass spectrometry**

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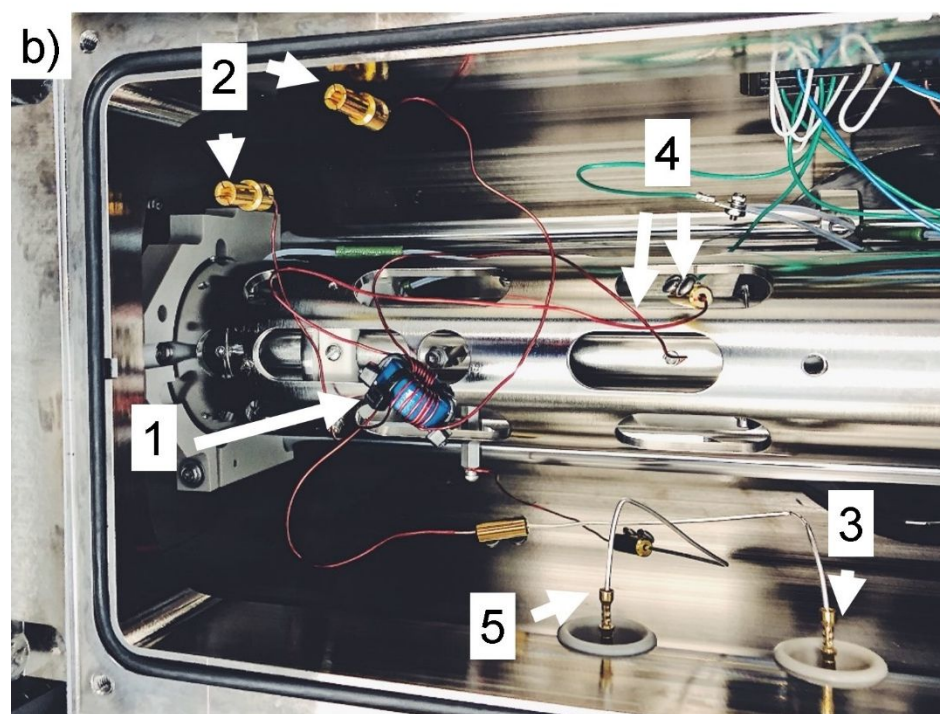
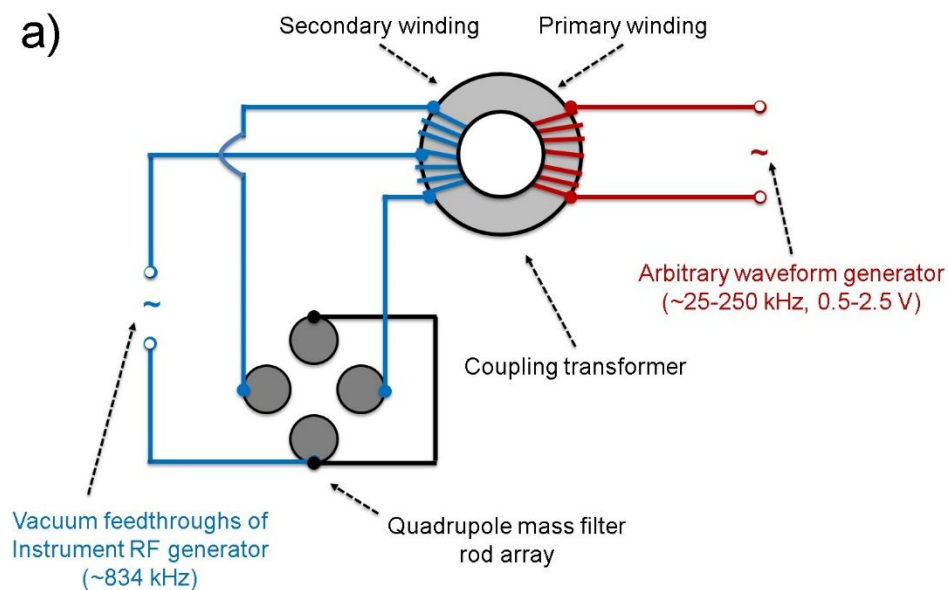
Figure S1: Coupling transformer for supplemental AC signal

Figure S2: Ion transmission verified before and after hardware modifications

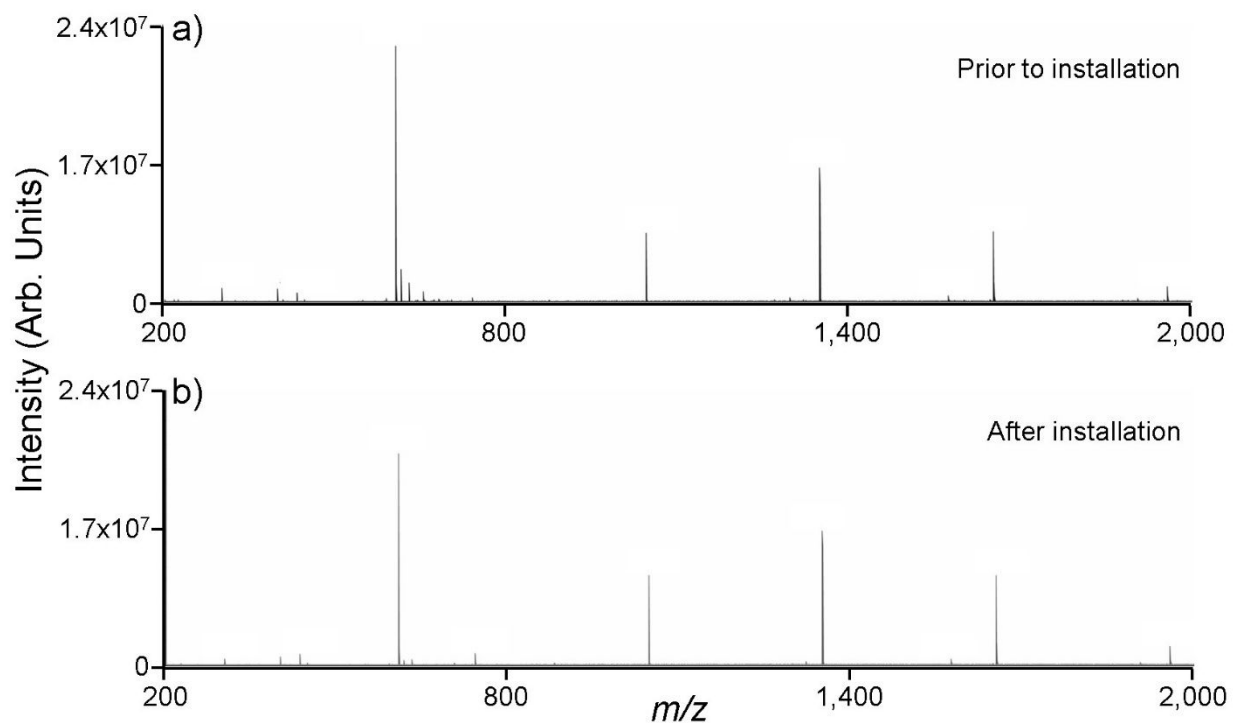
Figure S3: S/N improvements with CASI

Figure S4: Imaging dynamic range improvements with CASI

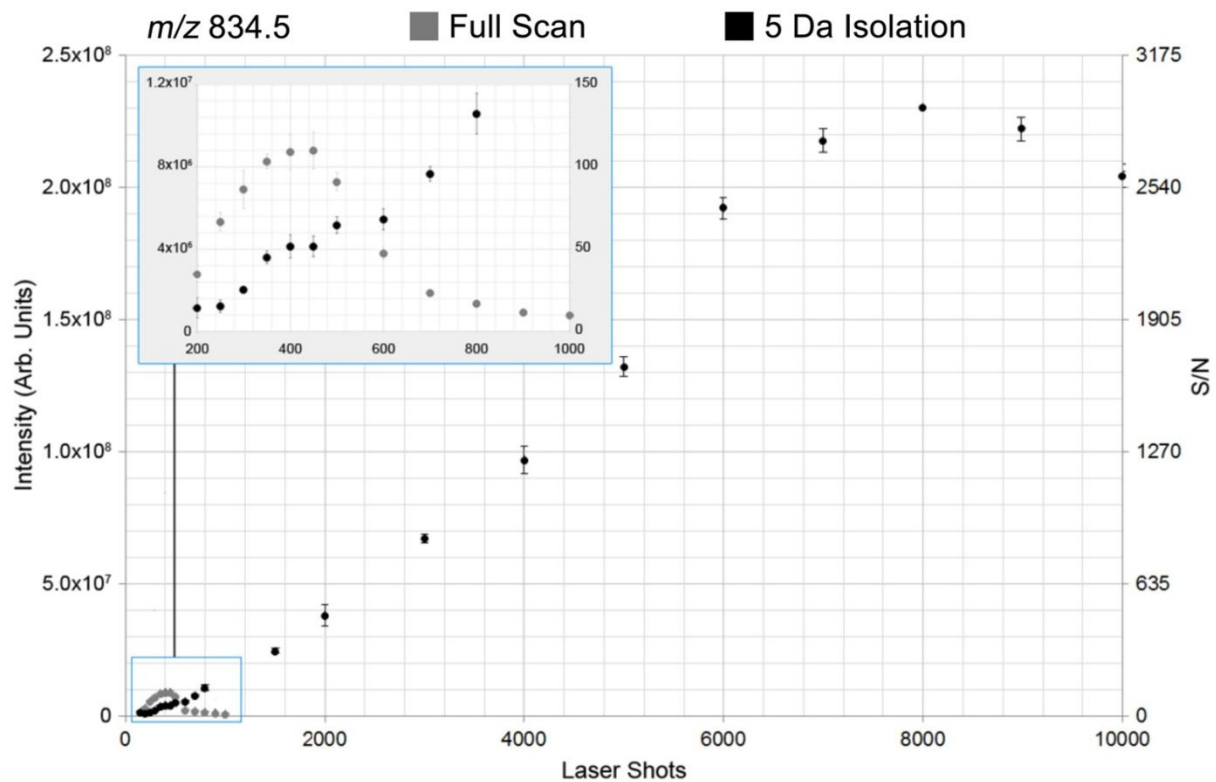
Figure S5: Imaging sensitivity and dynamic range improvements with SIE



**Figure S1:** a) Schematic of the transformer used to couple the arbitrary waveform generator signal onto the quadrupole mass filter. b) Photograph of the setup. Visible are the (1) transformer, (2) primary winding connections, (3) center tap connection to the instrument RF circuit, (4) secondary winding connections to the quadrupole rod pair, and (5) the unmodified instrument RF connection to the opposing rod pair.



**Figure S2:** Negative ion mode signal derived from an Agilent tune mix solution is nearly identical a) prior to and b) following installation of the coupling transformer. This ensures that no detrimental effects on ion transmission efficiency have been caused by the hardware modifications. This was also confirmed in positive ion mode (data not shown).



**Figure S3:** The intensity and S/N of  $m/z$  834.529 from a rat brain lipid extract is shown as a function of the number of MALDI laser shots for both the full scan and CASI (5 Da isolation window) acquisition modes. Each measurement represents four averages.

m/z	CASI				Full Scan			
	Min	Max	Avg	SD	Min	Max	Avg	SD
808.511	4	219	79	32	0	78	13	10
810.683	0	166	61	33	0	23	1.3	1.7
816.555	0	179	100	36	0	71	7.5	7.8
816.576	0	168	54	35	0	75	6.5	8.4
818.571	0	171	66	34	0	69	5.8	9.3
834.51	1	232	74	39	0	18	3	2.7
834.529	0	170	85	47	0	52	15	9.7
835.532	0	169	81	45	0	36	13	7.7
836.545	0	168	79	42	0	59	12	9
836.528	16	181	116	23	0	82	24	13
857.518	0	170	104	39	0	81	24	13

**Figure S4:** Improvements in dynamic range upon CASI as measured by minimum, maximum, average, and standard deviation of pixel intensities. Measurements were made by analyzing rainbow color intensity scale ion images using ImageJ (<https://imagej.nih.gov/ij/>, National Institutes of Health, Bethesda, MD).

m/z	Average Intensity			SIE				Full Scan			
	SIE	Full Scan	Fold-Change	Min	Max	Avg	SD	Min	Max	Avg	SD
<b>699.498</b>	1.4x10 <sup>7</sup>	4.2x10 <sup>6</sup>	3.3	6	74	39	15	3	23	13	4.2
<b>762.502</b>	1.1x10 <sup>7</sup>	3.5x10 <sup>6</sup>	3.1	1	86	32	212	0	29	10	6.6
<b>772.585</b>	3.6x10 <sup>6</sup>	9.0x10 <sup>5</sup>	4.0	0	56	27	10	0	16	7.3	3.4
<b>774.543</b>	2.9x10 <sup>7</sup>	6.9x10 <sup>6</sup>	4.2	1	125	50	30	0	30	12	6.7
<b>794.570</b>	4.7x10 <sup>6</sup>	1.5x10 <sup>6</sup>	3.1	0	126	37	20	0	41	12	7.4
<b>804.530</b>	2.8x10 <sup>6</sup>	1.0x10 <sup>6</sup>	2.8	0	104	30	29	0	44	11	12
<b>816.570</b>	4.6x10 <sup>5</sup>	7.0x10 <sup>4</sup>	6.6	0	119	18	22	0	36	3.5	6.5
<b>850.572</b>	8.0x10 <sup>6</sup>	2.5x10 <sup>6</sup>	3.2	0	61	21	15	0	20	7.2	5.1
<b>856.510</b>	1.6x10 <sup>7</sup>	4.0x10 <sup>6</sup>	4.0	0	75	30	19	0	18	7.6	4.4
<b>860.592</b>	6.5x10 <sup>6</sup>	2.2x10 <sup>6</sup>	3.0	0	100	33	29	0	36	12	10
<b>916.653</b>	5.6x10 <sup>6</sup>	1.8x10 <sup>6</sup>	3.1	0	81	27	22	0	28	10	7.6
<b>934.666</b>	7.2x10 <sup>6</sup>	1.8x10 <sup>6</sup>	4.0	0	110	43	3.1	0	29	12	8.5

**Figure S5:** Improvements in sensitivity and dynamic range upon SIE as measured by average ion intensities (*i.e.*, sensitivity) and minimum, maximum, average, and standard deviation of pixel intensities (*i.e.*, dynamic range). The dynamic range measurements were made by analyzing monochromatic ion images using ImageJ (<https://imagej.nih.gov/ij/>, National Institutes of Health, Bethesda, MD).