Electronic Supplementary Material (ESI) for Analytical Methods.

Repeatability and reproducibility of desorption electrospray ionization-mass spectrometry (DESI-MS) for the imaging analysis of human cancer tissue: a gateway for clinical applications

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Supplementary file 1: DESI-MS sprayer construction

A schema of the home-built DESI-MS sprayer is shown in Fig. 1a. A Swagelok T element is used as the main body for the assembly of two fused silica capillaries held in place by sleeves and ferrules for the simultaneous delivery of gas and solvent.

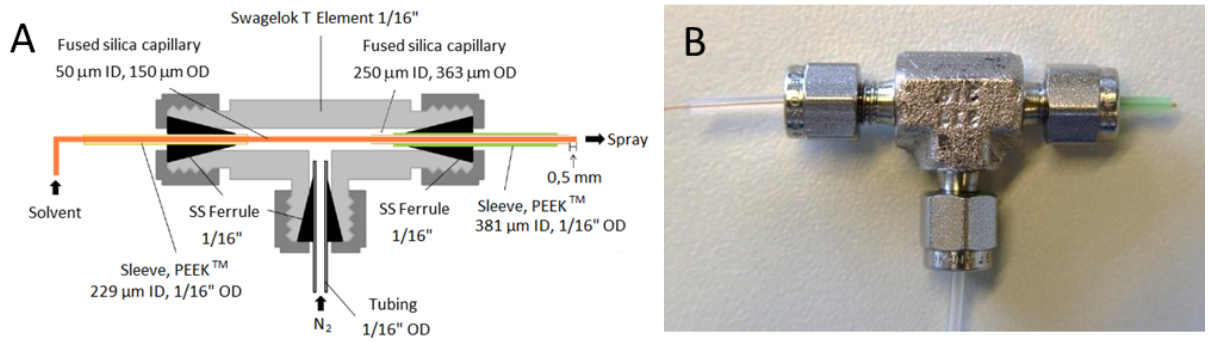


Figure S-1: A) Scheme and B) photograph of a DESI-MS sprayer as used in this study

A 50 µm ID, 150 µm OD fused silica capillary was used to deliver the solvent and inserted into a 1.5 cm long 250 µm ID, 363 µm OD fused silica capillary delivering the nebulising gas. Since perfect coaxial orientation of the two fused silica capillaries proved difficult to achieve, the two capillaries were oriented with the inner capillary touching the outer capillary at its top edge when the sprayer is directed on the sample (see Fig. X). This was associated with an elliptical spray point facing towards the inlet capillary of the mass spectrometer and an increased ion yield. The polyimide coating of both capillaries was removed to enable accurate shaping of the fused silica capillaries using a ceramic waver. This was found to be crucial for obtaining a stable and symmetrical electrospray, which in turn was essential for image quality and ion yield. Best image quality was found with a spray head featuring the solvent capillary extruding approximately 0.5 mm from the gas capillary.

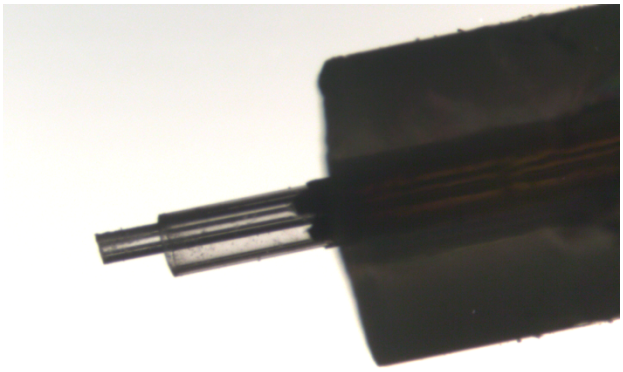
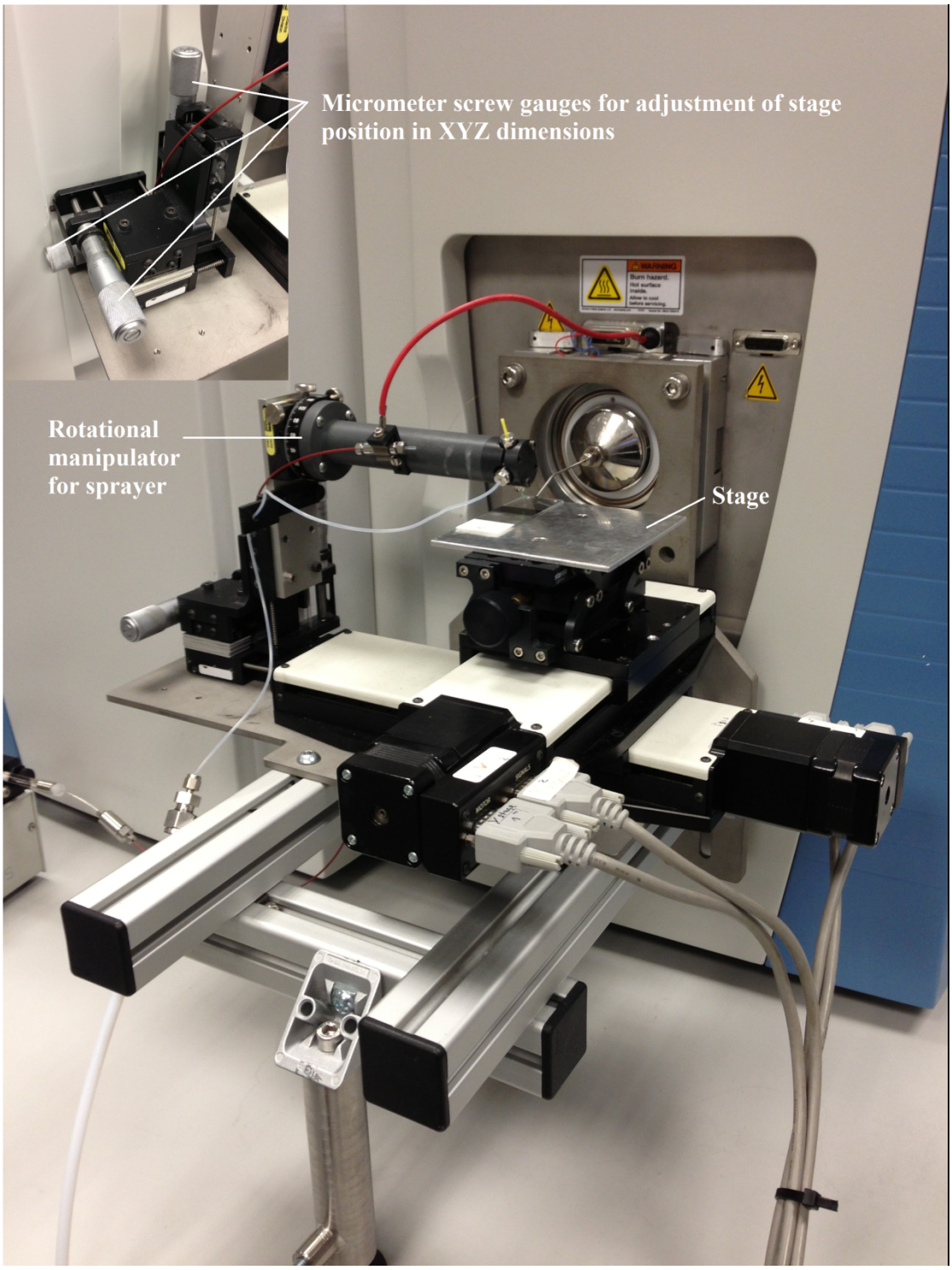


Figure S-2: Photograph of the DESI-MS spray head used in this study; 5x magnification

Supplementary file 2: Home-built 3D XYZ integrated DESI-MS Stage



Supplementary file 3: Mean CVs of 65 lipid peaks

Single samples taken together	16±7%
Quadrants taken together	22±7%
Full dataset	30±14%

	Single	Quads	Full
I 1_1	25%	26%	30%
I 1_2	26%		
I 1_3	13%		
I 1_4	10%		
I 2_1	15%	23%	
I 2_2	8%		
I 2_3	19%		
I 2_4	22%		
I 3_1	20%	23%	
I 3_2	18%		
I 3_3	19%		
I 3_4	10%		
I 4_1	11%	15%	
I 4_2	11%		
I 4_3	10%		
I 4_4	10%		

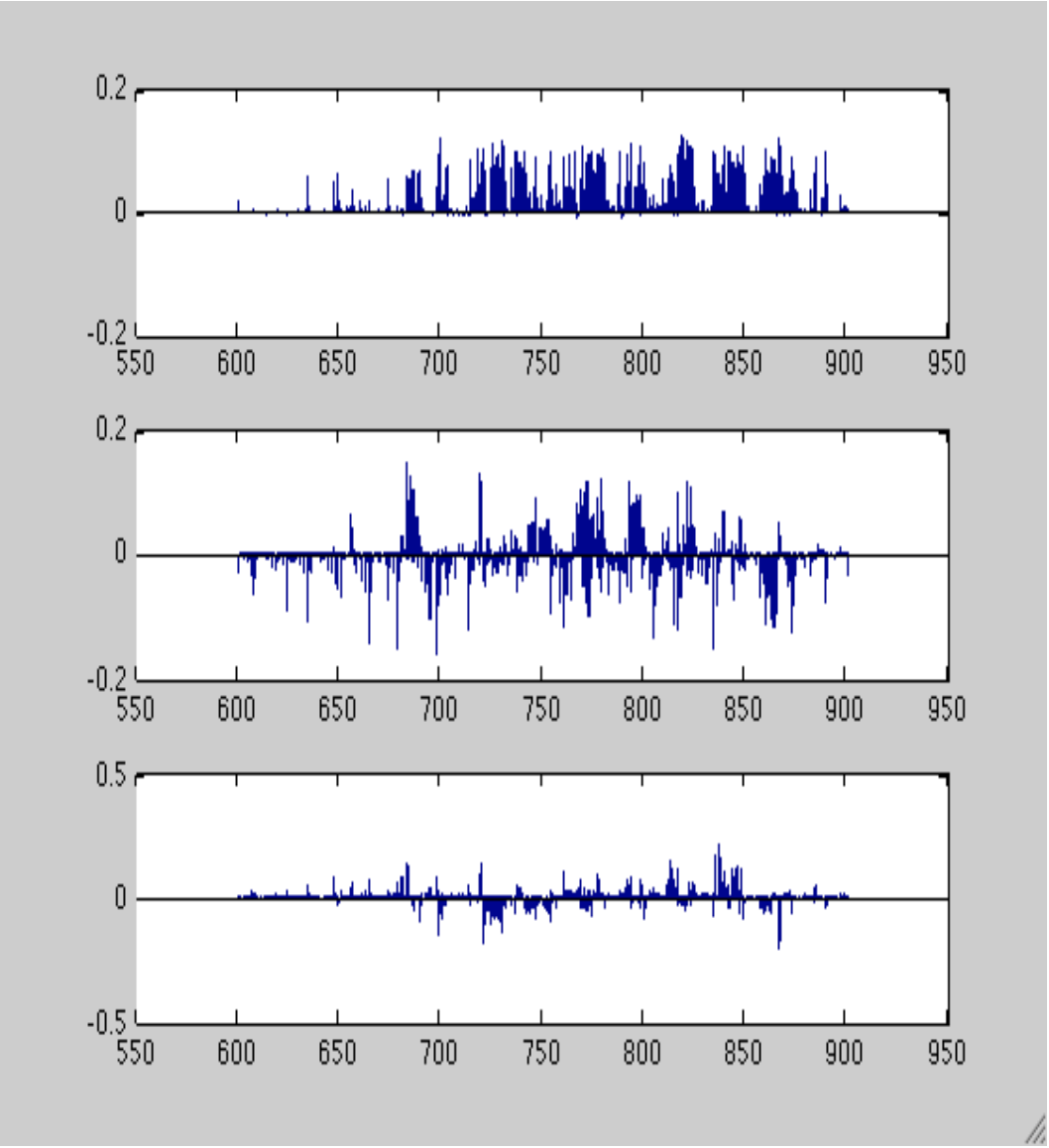
Individual lipid peaks

Tentative lipid identities were determined using full resolution m/z values and searching the Lipidmaps (www.lipimaps.org) structure database (LMSD) for M-H ions with a mass tolerance of 5ppm. The 27 lipid species used for the purpose of reproducibility measurements are marked by an asteriks.

Observed mass	Expected	Tentative assignment	CV
700.53*	700.52866	PE(P-34:1)	19%
701.51*	701.51268	PA(36:1)	18%
703.52	703.52833	PA(36:0)	51%
714.51	714.50793	PE(34:2)	38%
716.53*	716.52358	PE(34:1)	21%
718.54	718.53923	PE(34:0)	16%
720.5	720.49736	PE(P-36:5)	27%
724.53	724.52866	PE(P-36:3)	21%
728.56*	728.55996	PE(P-36:1)	16%
730.58	730.57561	PE(P-36:0)	37%
736.53	736.52866	Unassigned	29%
738.51	738.50793	PE(36:4)	25%
740.52	740.52358	PE(36:3)	23%
742.54*	742.53923	PE(36:2)	10%
744.56	744.55488	PE(36:1)	8%
746.51	746.51301	PE(P-38:6)	9%
747.52	747.51816	PG(34:1)	18%
748.53*	748.52866	PE(P-38:5)	14%

750.55*	750.54431	PE(P-38:4)	7%
752.55	752.55996	PE(P-38:3)	9%
762.51	762.50793	PE(38:6)	30%
764.53*	764.52358	PE(38:5)	24%
766.54	766.53923	PE(38:4)	9%
768.55	768.55488	PE(38:3)	9%
769.51	769.50251	PG(36:4)	15%
770.57*	770.57053	PE(38:2)	18%
771.52	771.51816	PG(36:3)	9%
772.55	772.54979	PS(P-36:1)	13%
772.59	772.58618	PE(38:1)	13%
773.54	773.53381	PG(36:2)	9%
775.55	775.54946	PG(36:1)	11%
777.56	777.56511	PG(36:0)	31%
778.58*	778.57561	PE(P-40:4)	14%
786.53	786.52906	PS(36:2)	21%
788.55	788.54471	PS(36:1)	7%
790.56	790.56036	PS(36:0)	8%
792.56*	792.55488	PE(40:5)	10%
793.5	793.50251	PG(38:6)	16%
794.57*	794.57053	PE(40:4)	8%
795.52	795.51816	PG(38:5)	8%
795.58*	795.59093	Unassigned	8%
796.55	796.54979	PS(P-38:3)	12%
797.65	797.6542	Unassigned	12%
799.67	799.66985	Unassigned	49%
810.53*	810.52906	PS(38:4)	19%
812.55	812.54471	PS(38:3)	15%
816.58	816.57601	PS(38:1)	13%
819.52	819.51816	PG(40:7)	16%
820.56		Unassigned	10%
822.58	822.60183	PE(42:4)	22%
833.52	833.51855	PI(34:2)	35%
834.53	834.52906	PS(40:6)	26%
835.53	835.5342	PI(34:1)	21%
838.56	838.56036	PS(40:4)	16%
844.61	844.60731	PS(40:1)	18%
846.66	846.65934	PS(O-41:0)	26%
847.65	847.64336	Unassigned	32%
857.52*	857.51855	PI(36:4)	18%
861.55*	861.54985	PI(36:2)	10%
863.57	863.5655	PI(36:1)	29%
865.51	865.50251	PG(44:12)	30%
872.64	871.64336	PG(43:2)	28%
883.54*	883.5342	PI(38:5)	10%
885.55*	885.54985	PI(38:4)	6%
887.56*	887.5655	PI(38:3)	7%

Section S4: Loading plots



Loading plots for first three principal components. Data has undergone median fold change normalisation and log transformation. Due to the similarity of the spectra the first principal differentiates the spectra on signal intensity of all species.