Electronic Supplementary Material (ESI) for Analyst

Supporting Information

Interactive hyperspectral approach for exploring and interpreting DESI-MS images of cancer and normal tissue sections

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A video illustrating the brushing procedure is available as electronic supplemental material. For further information, please contact the Corresponding Author.

EXPERIMENTAL SECTION

Tissue Handling (as reported in publications[1-4])

All tissue samples were handled in accordance with approved Institutional Review Board (IRB) protocols at the Indiana University School of Medicine. All patients from whom tissue was collected had voluntarily signed an informed consent and Health Insurance Portability and Accountability Act (HIPAA) documents prior to study participation, in accordance with the IRB protocol.

Fresh tissue samples were frozen in liquid nitrogen and stored in closed containers at -80°C until sliced into 15 µm thick sections using a Shandon SME Cryotome cryostat (GMI, INC., Ramsey, MN, USA), and thaw-mounted onto glass slides. All slides contained one section of tumor tissue and one section of adjacent normal tissue from the same patient. The slides were stored at -80°C and prior to analysis were allowed to come to room temperature and then dried under nitrogen in a dessicator for approximately 20 min. All the samples were sequentially subjected to DESI-MS imaging analysis. Sections serial to those used for DESI imaging were formalin fixed and stained using hematoxylin and eosin (H&E) for pathological examination.

DESI-MS 2D imaging (as reported in publications[1-4])

All experiments were carried out using a commercial LTQ linear ion trap mass spectrometer, with the automatic gain control turned off. The XCalibur 2.0 software (Thermo Fisher Scientific, San Jose, CA, USA) was used to control the instrument, and a lab-built prototype DESI ion source[5] was used to analyze the samples. The DESI spray was optimized to achieve a small and uniform spray spot on the sample surface for minimal splashing[6], assuring high quality images. The 2D moving stage included an XYZ integrated linear stage (Newport, Richmond, CA, USA) and a rotary stage (Parker Automation, Irwin, PA, USA). The DESI spray was positioned at a vertical distance of 2 mm from the sample surface and at an incident angle of 52°. The spray solvent for MS acquisition was acetonitrile:water (50:50 v/v) with a 5 kV spray voltage applied. Acetonitrile was purchased from Sigma-Aldrich (St. Louis, MO, USA) and water (18.2 MΩ·cm) was purified by a PureLab ultra system by Elga LabWater (High Wycombe, UK). The nitrogen gas pressure was 150 psi and the solvent flow rate was 1.5 µL/min. The tissue sections were scanned using the 2D moving stage in horizontal rows separated by a

250 µm vertical step until the entire tissue sample has been assayed. According to the acquisition time of the mass spectrum (about 1.14 sec) and the dimensions of each sample, a different number of horizontal and vertical pixels were obtained. Full scan mass spectra were acquired in negative ion mode in the mass range *m/z* 150–1000 for germ samples and *m/z* 200–1000 for the other ones.

Table S1. Description of the tissue sample pairs used in this study. Table includes pathological diagnosis of the two sections analyzed (annotated as left and right sections in glass slide) and comments of the multivariate analysis results.

Patient case codes	**Human tissue type**	**Pathological Diagnosis and Comments**
MH0204_33	kidney	*Pathology:* Papillary RCC (left) and normal (right) tissue
UH0505_12	kidney	*Pathology:* Papillary RCC (left) and normal (right) tissue. *Comments:* Tumor section had normal tissue with inflammatory cells, highlighted as normal tissue by PCA
UH0710_33	kidney	*Pathology:* Papillary RCC (left) and normal (right) tissue. *Comments:* Only separation between tumor tissue section and background/normal tissue section was observed by PCA
UH9610_15	kidney	*Pathology:* Papillary RCC (left) and normal (right) tissue. *Comments:* Tumor section had normal tissue, correctly highlighted as normal tissue by PCA
UH9812_03	kidney	*Pathology:* Papillary RCC (left) and normal (right) tissue
UH9905_18	kidney	*Pathology:* Papillary RCC (left) and normal (right) tissue. *Comments:* Tumor section had normal tissue, correctly highlighted as normal tissue by PCA
UH9911_05	kidney	*Pathology:* Papillary RCC (left) and normal (right) tissue
UH9912_01	kidney	*Pathology:* Papillary RCC (left) and normal (right) tissue

UH9911_09	kidney	*Pathology:* Papillary RCC (left) and normal (right) tissue. *Comments:* Tumor section had normal tissue, correctly highlighted as normal tissue by PCA
MH0201_33	kidney	*Pathology:* Clear cell RCC (left) and normal (right) tissue. *Comments:* Separation between tumor and normal tissue sections was observed in the PC1-4 space
MH0111_10	kidney	*Pathology:* Clear cell RCC (left) and normal (right) tissue
MH0204_06	kidney	*Pathology:* Clear cell RCC (left) and normal (right) tissue
MH0111_55	kidney	*Pathology:* Clear cell RCC (left) and normal (right) tissue
MH0111_45	kidney	*Pathology:* Clear cell RCC (left) and normal (right) tissue
MH0110_28	kidney	*Pathology:* Clear cell RCC (left) and normal (right) tissue. *Comments:* Only separation between background and the tissue sections was observed by PCA
MH0108_13	kidney	*Pathology:* Clear cell RCC (left) and normal (right) tissue. *Comments:* Only separation between background and the tissue sections was observed by PCA
UH0003_15	bladder	*Pathology:* cancer (left) and normal (right) tissue
UH0004_36	bladder	*Pathology:* cancer (left) and normal (right) tissue
UH0005_30	bladder	*Pathology:* cancer (right) and normal (left) tissue. *Comments:* Normal section had urothelium carcinoma in situ, correctly highlighted as tumor tissue by PCA
UH0007_19	bladder	*Pathology:* cancer (left) and normal (right) tissue

UH0010_19	bladder	*Pathology:* cancer (right) and normal (left) tissue
UH0012_03	bladder	*Pathology:* cancer (left) and normal (right) tissue. *Comments:* Only separation between tumor tissue section and background/normal tissue section was observed by PCA
UH0103_23	bladder	*Pathology:* cancer (left) and normal (right) tissue
UH0112_37	bladder	*Pathology:* cancer (left) and normal (right) tissue
UH0201_06	bladder	*Pathology:* cancer (left) and normal (right) tissue
UH0201_49	bladder	*Pathology:* cancer (left) and normal (right) tissue. *Comments:* Incomplete separation between tumor and normal tissue section was observed by PCA
UH0208_18	bladder	*Pathology:* cancer (left) and normal (right) tissue
UH0504_65	bladder	*Pathology:* cancer (left) and normal (right) tissue. *Comments:* Tumor tissue had normal tissue. Incomplete separation between the tissue sections was observed by PCA
UH0712_18	bladder	*Pathology:* both sections are normal tissue. *Comments:* Correctly, no separation between the tissue sections was observed by PCA
UH0001_02	germ	*Pathology:* seminoma (left) and normal tubules (right) tissue
UH0101_35	germ	*Pathology:* seminoma (left) and normal tubules (right) tissue
UH0108_13	germ	*Pathology:* seminoma (left) and normal tubules (right) tissue. *Comments:* Part of the normal tissue was erroneously highlighted as tumor tissue by PCA
UH0201_09	germ	*Pathology:* seminoma (left) and normal tubules (right) tissue

UH0201_20	germ	*Pathology:* seminoma (left) and normal fat/muscles (right) tissue
UH0206_08	germ	*Pathology:* seminoma (left) and normal tubules (right) tissue. *Comments:* Normal section had tumor tissue. No clear separation between the tissue sections was observed by PCA
UH0711_36	germ	*Pathology:* seminoma (left) and normal tubules (right) tissue
UH0503_08	germ	*Pathology:* seminoma (right) and normal tubules (left) tissue
UH0209_33	germ	*Pathology:* seminoma (left) and normal tubules (right) tissue
G1_03	germ	*Pathology:* seminoma (left) and normal tubules (right) tissue
G1_07	germ	*Pathology:* seminoma (left) and normal fat/muscles (right) tissue
MH0107_01	prostate	*Pathology:* cancer (left) and normal (right) tissue. *Comments:* Small part of the normal section had tumor, highlighted as tumor by PCA
MH0108_32	prostate	*Pathology:* cancer (left) and normal (right) tissue
MH0205_17	prostate	*Pathology:* normal (left) and normal with PIN (right) tissue
UH0002_25	prostate	*Pathology:* cancer (left) and normal (right) tissue
MH0301_17	prostate	*Pathology:* cancer (left) and normal (right) tissue
MH0212_04	prostate	*Pathology:* mostly cancer (left) and normal with small amount of PIN (right) tissue

UH0002_20	prostate	*Pathology:* cancer (left) and normal with small amount of PIN (right) tissue
UH0003_12	prostate	*Pathology:* mostly PIN (left) and normal (right) tissue. *Comments:* Incomplete separation between the tissue sections was observed by PCA

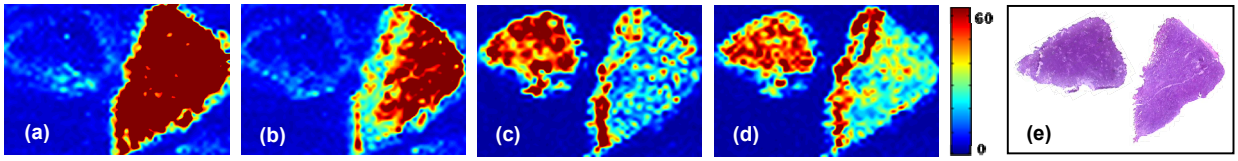

Figure S1. Negative ion mode imaging of kidney tissue including areas of papillary RCC and adjacent normal tissue of sample UH9812_03. (a) Ion image of *m/z* 217.2. (b) Ion image of *m/z* 271.2. (c) Ion image of *m/z* 788.5. (d) Ion image of *m/z* 810.5. (e) H&E stained tissue sections of the tumor tissue (left) and normal tissue (right).

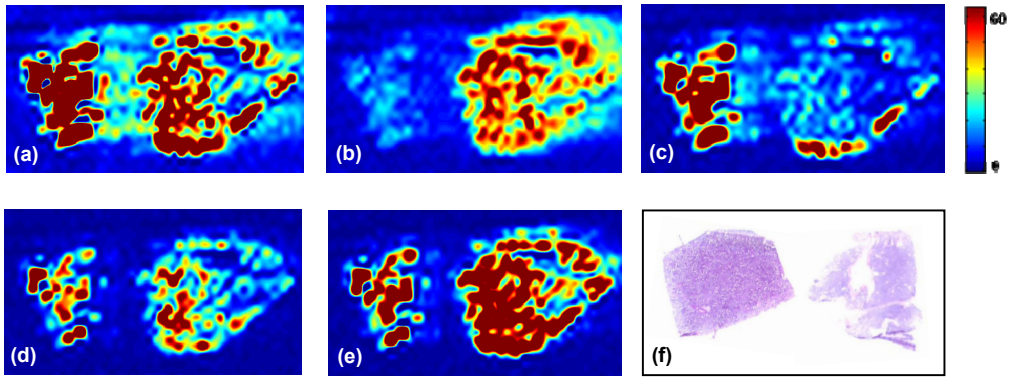

Figure S2. Negative ion mode imaging of kidney tissue including areas of clear cell RCC and adjacent normal tissue of sample MH0111_10. (a) Ion image of *m/z* 281.5. (b) Ion image of *m/z* 303.2. (c) Ion image of *m/z* 562.9. (d) Ion image of *m/z* 810.2. (e) Ion image of *m/z* 885.5. (f) H&E stained tissue sections of the tumor tissue (left) and normal tissue (right).

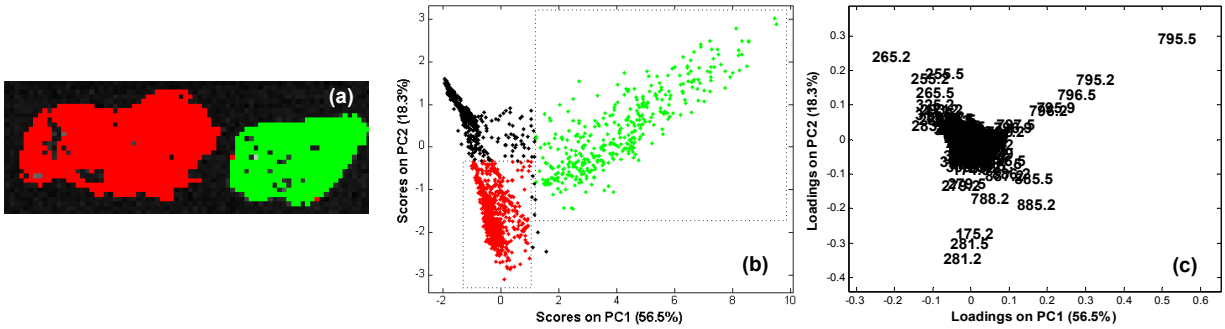

Figure S3. PCA for germ sample UH0201_09. (a) Correspondence (indicated by matching colors) between PC scores selected by means of the brushing procedure and pixels in the image space (red: tumor section, green: normal section). (b) PC1 (56.5% of the total variance) vs. PC2 (18.3% of the total variance) score plot. (c) PC1 vs. PC2 loading plot labeled in terms of *m/z* ratios.

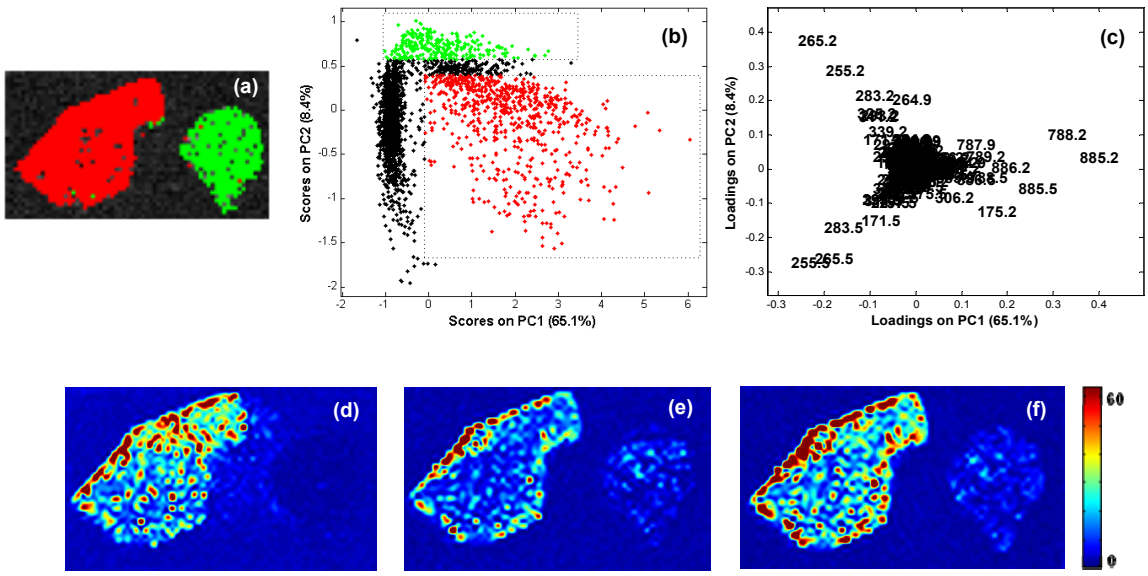

Figure S4. PCA for germ sample UH0201_20. (a) Correspondence (indicated by matching colors) between PC scores selected by means of the brushing procedure and pixels in the image space (red: tumor section, green: normal section). (b) PC1 (65.1% of the total variance) vs. PC2 (8.4% of the total variance) score plot. (c) PC1 vs. PC2 loading plot labeled in terms of *m/z* ratios. (d) Ion image of *m/z* 175.2. (e) Ion image of *m/z* 788.5. (f) Ion image of *m/z* 885.5.

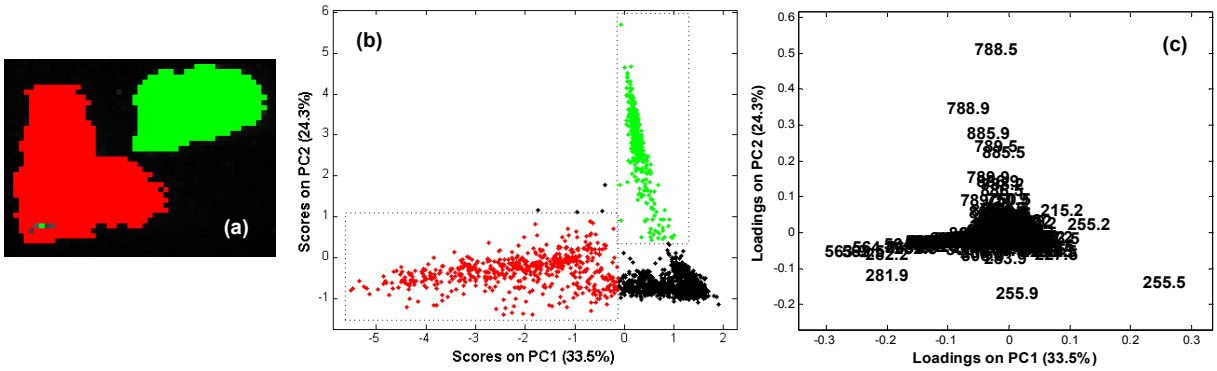

Figure S5. PCA for bladder sample UH0208_18. (a) Correspondence (indicated by matching colors) between PC scores selected by means of the brushing procedure and pixels in the image space (red: tumor section, green: normal section). (b) PC1 (33.5% of the total variance) vs. PC2 (24.3% of the total variance) score plot. (c) PC1 vs. PC2 loading plot labeled in terms of *m/z* ratios.

References for Supporting Information

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