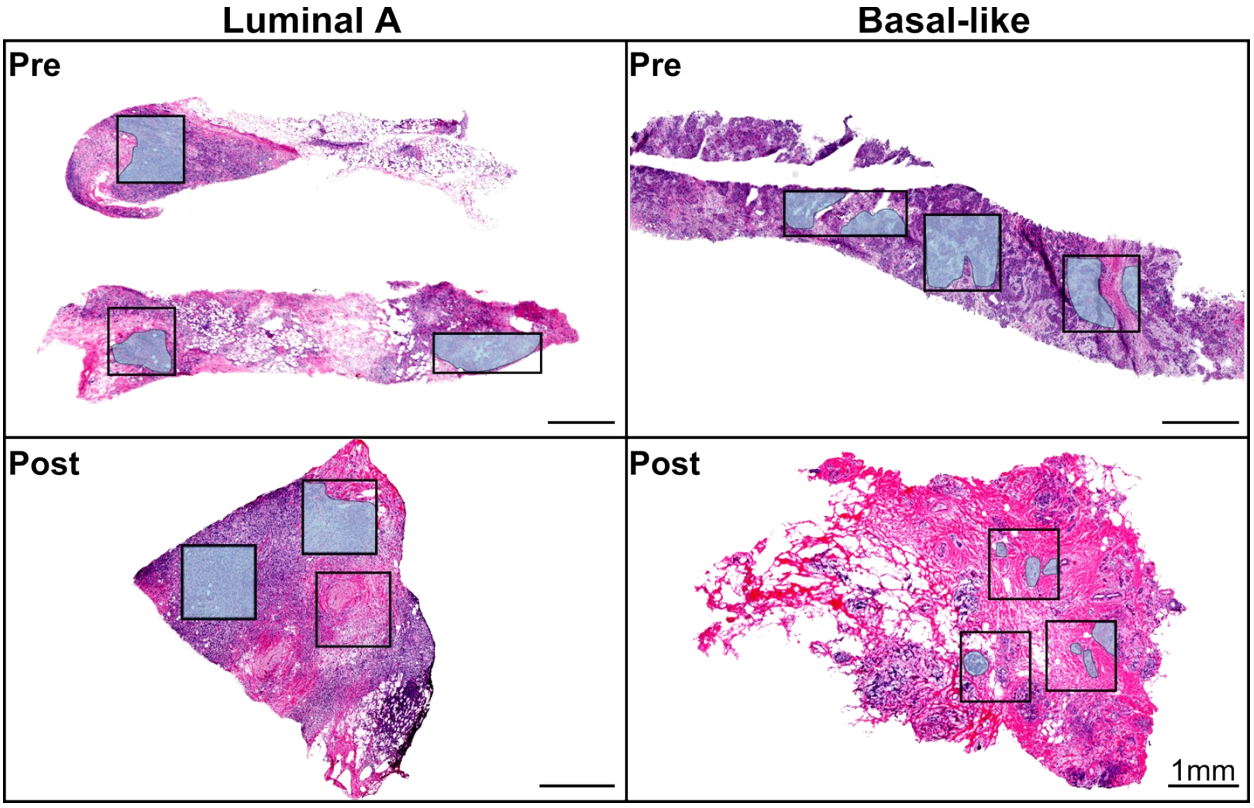
Electronic Supplementary Material (ESI) for Analyst.

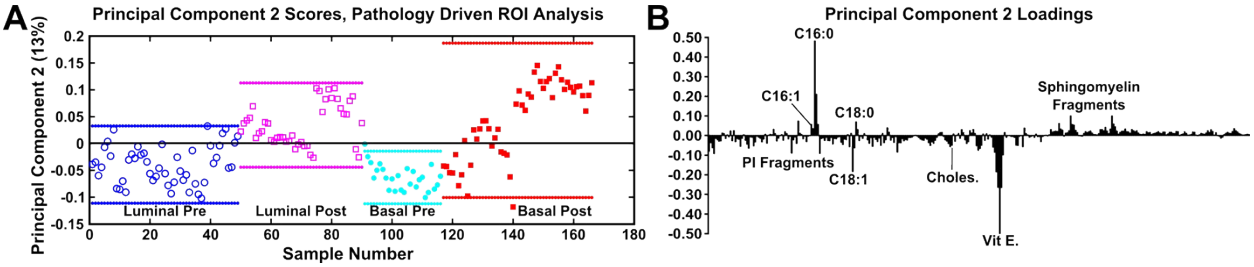
Supplementary Table 1. Key negative and positive ion *m/z* and fragment identification for peaks observed in PCA.[1]

Negative Ions			
Mass (m/z)	**Composition**	**Deviation (ppm)**	**Possible Biomolecule**
26.00	CN^-	17.50	--
41.00	CNO^-	14.10	--
62.96	PO_2^-	-20.65	--
79.97	PO_3H^-	-20.01	--
163.07	$C_{10}H_{11}O_2^-$	-3.68	Vitamin E fragment
227.20	$C_{14}H_{27}O_2^-$	-1.36	Myristic acid, 14:0
241.01	$C_6H_{10}PO_8^-$	-27.80	Phosphoinositol fragment
253.22	$C_{16}H_{29}O_2^-$	-1.97	Palmitoleic acid; 16:1
255.23	$C_{16}H_{31}O_2^-$	5.49	Palmitic acid; 16:0
259.02	$C_6H_{12}PO_9^-$	-31.27	Phosphoinositol fragment
279.24	$C_{18}H_{29}O_2^-$	-14.68	Linoleic acid; 18:2
281.25	$C_{18}H_{33}O_2^-$	-7.11	Oleic acid; 18:1
283.26	$C_{18}H_{35}O_2^-$	-14.47	Stearic acid; 18:0
299.05	$C_9H_{16}PO_9^-$	-31.47	Phosphoinositol fragment
303.24	$C_{20}H_{31}O_2^-$	9.89	Arachidonic acid; 20:4
385.35	$C_{27}H_{45}O^-$	-3.63	Cholesterol
429.37	$C_{29}H_{49}O_2^-$	-9.78	Vitamin E α-tocopherol
616.5	$C_{34}H_{67}NO_6P^-$	-31.31	SM(34:1)
642.5	$C_{36}H_{69}NO_6P^-$	-32.69	SM(34:1)
687.6	$C_{38}H_{76}N_2O_6P^-$	-38.59	SM(34:1)

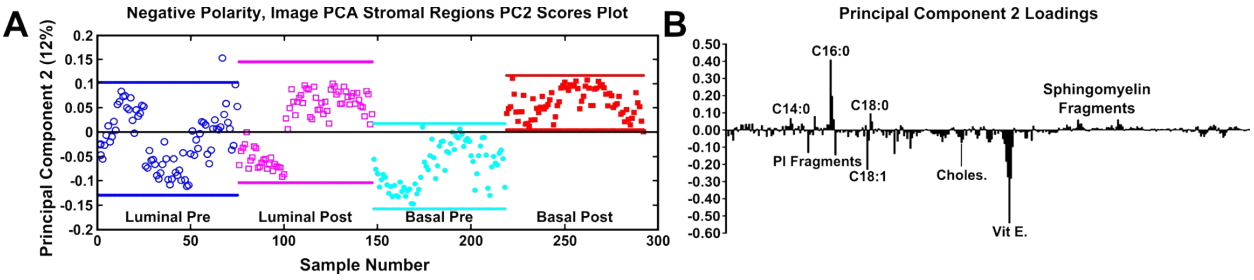
Positive Ions			
Mass (m/z)	**Composition**	**Deviation (ppm)**	**Possible Biomolecule**
206.06	$C_5H_{14}NPO_4Na^+$	25.24	Phosphatidylcholine fragment
224.10	$C_8H_{19}PNO_4^+$	29.45	Phosphatidylcholine fragment
246.09	$C_8H_{18}PNO_4Na^+$	15.85	Phosphatidylcholine fragment
311.26	$C_{19}H_{35}O_3^+$	9.32	MAG 16:1
313.27	$C_{19}H_{37}O_3^+$	8.94	MAG 16:0
337.27	$C_{21}H_{37}O_3^+$	9.49	MAG 18:2
339.29	$C_{21}H_{39}O_3^+$	6.19	MAG 18:1
369.35	$C_{27}H_{45}^+$	-2.17	Cholesterol
430.37	$C_{29}H_{50}O_2^+$	14.64	Vitamin E
549.50	$C_{35}H_{65}O_4^+$	-11.28	DAG 32:1
551.50	$C_{35}H_{67}O_4^+$	11.97	DAG 32:0
575.52	$C_{37}H_{67}O_4^+$	0.70	DAG 34:2
577.52	$C_{37}H_{69}O_4^+$	20.78	DAG 34:1
601.52	$C_{39}H_{69}O_4^+$	13.13	DAG 36:3
603.53	$C_{39}H_{71}O_4^+$	20.05	DAG 36:2



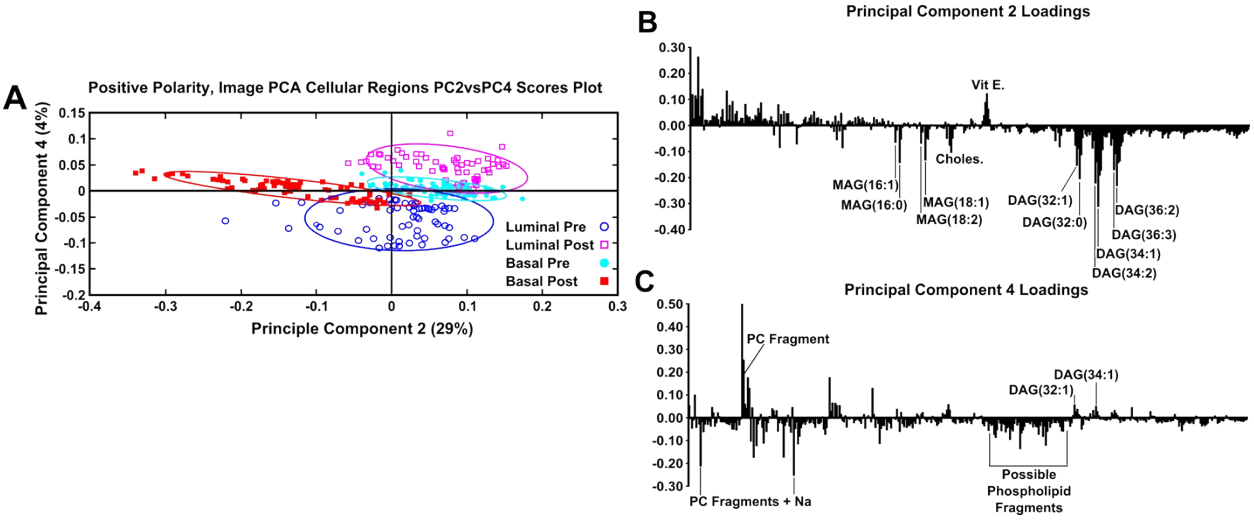
Supplementary Figure 1. Optical H&E images showing the pathologist directed ROIs in blue. These regions were reconstructed and compared using spectral PCA for the results presented in Supplementary Figure 2. All scale bars represent 1 mm.



Supplementary Figure 2. (A) PC2 scores generated from pathology driven analysis by tile removal for ion *m/z* > 200. PC2 shows an overall variance of 13%. (B) PC2 loadings displaying chemical species that correspond to scores from pathology driven analysis. Pre-chemotherapeutic tissues shown as blue colored ○ and cyan ●, post-chemotherapeutic tissues shown as colored magenta □ and red ■.



Supplementary Figure 3. Spectral PCA results of negative polarity stromal areas between tissue samples using image PCA masks to reconstruct only stromal regions for each tissue. (A) PC2 scores generated from PCA masks using negative ions *m/z* > 200. (B) Loadings plot displaying the chemical species that correspond to scores from PCA mask analysis. The heterogeneity of the stromal regions can be seen within the figure, possibly denoting that stromal regions can change when located near dense tumor regions. Pre-chemotherapeutic tissues shown as blue colored ○ and cyan ●, post-chemotherapeutic tissues shown as colored magenta □ and red ■.



Supplementary Figure 4. Spectral PCA results of positive polarity cellular/tumor areas between tissue samples using image PCA masks to reconstruct only cellular/tumor regions for each tissue. (A) PC2 vs PC4 scores using image PCA masks to reconstruct the cellular/tumor regions using positive ions *m/z* > 200. (B) PC2 loadings plot displaying the chemical species that correspond to PC2 scores(x-axis) (C) PC4 loadings plot displaying the chemical species that correspond to PC4 scores (y-axis). Pre-chemotherapeutic tissues shown as blue colored ○ and cyan ●, post-chemotherapeutic tissues shown as colored magenta □ and red ■.

[1] **M. K. Passarelli and N. Winograd, "Lipid imaging with time-of-flight secondary ion mass spectrometry (ToF-SIMS)," *Biochim Biophys Acta,* vol. 1811, pp. 976-90, Nov 2011.**