**Label-free Raman spectroscopy detects stromal adaptations in pre-metastatic lungs**

Santosh Kumar Paidia,1, Asif Rizwanb,1, Chao Zhenga,c,1, Menglin Chengb, Kristine Glundeb,d,2 and Ishan Barmana,d,2

aDepartment of Mechanical Engineering, Johns Hopkins University, Baltimore, Maryland, USA

bDivision of Cancer Imaging Research, The Russell H. Morgan Department of Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

cDepartment of Breast Surgery, The Second Hospital of Shandong University, Jinan, Shandong, China

dThe Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

1These authors contributed equally

2To whom correspondence may be addressed. E-mail: [ibarman@jhu.edu](mailto:ibarman@jhu.edu)or[kglunde@mri.jhu.edu](mailto:kglunde@mri.jhu.edu)

**Supplementary Information**

**Table ST1.** Band assignment for spectral features observed in PC loadings derived from the Raman spectra of the mouse lungs.

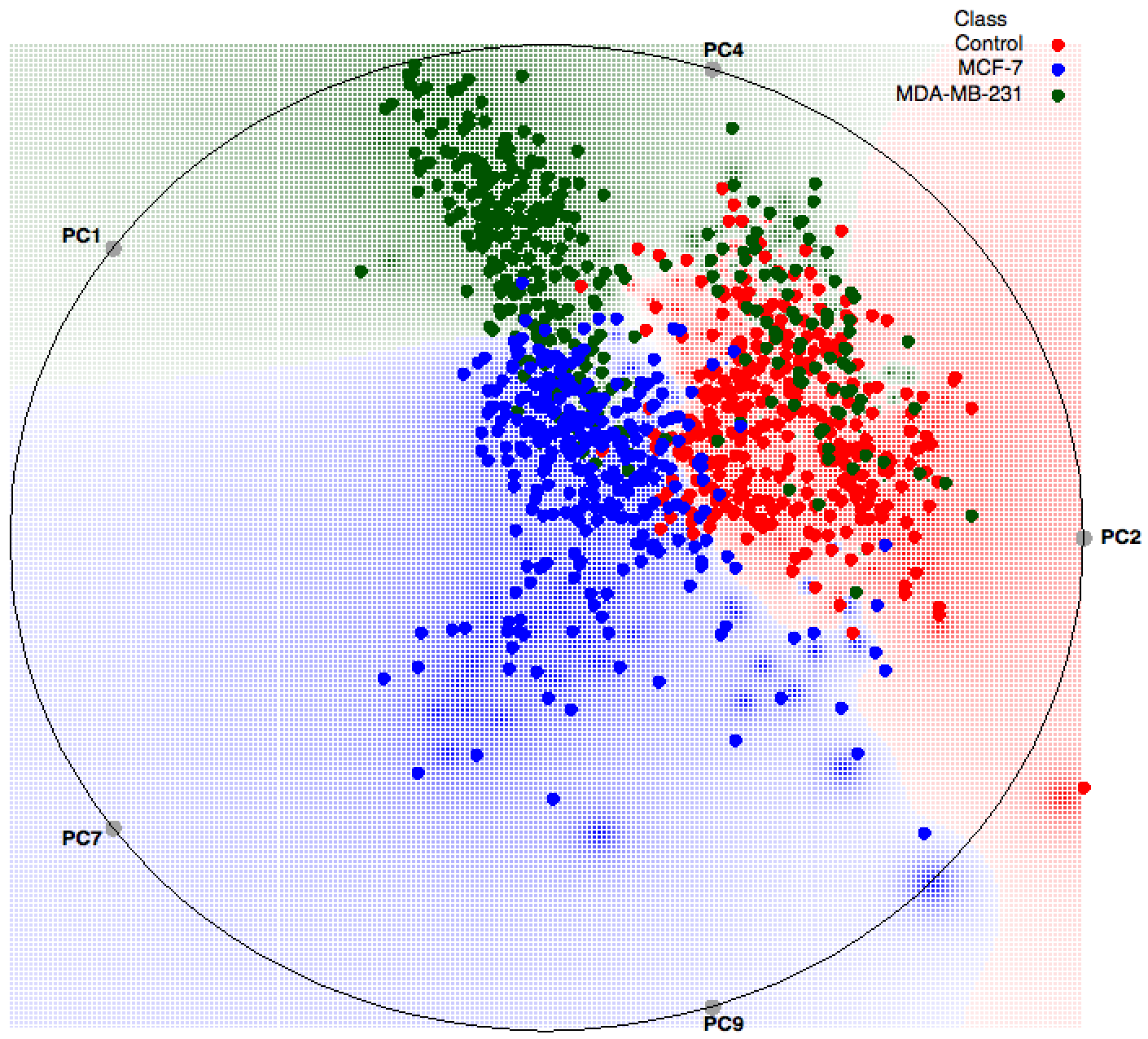
|  |  |
| --- | --- |
| **Observed Raman Peaks in the PC loadings (cm-1)** | **Raman band assignment from literature** |
| 859 | C-C stretch of proline (Collagen) |
| 917 | C-C stretch of proline ring (Collagen) |
| 1003 | C–C stretching vibration of the aromatic ring in the phenylalanine side chain (Collagen) |
| 1061 | OSO3 symmetric stretching (Proteoglycans) |
| 1304 | In-plane CH2 twisting modes of lipids |
| 1442 | CH2 deformation of lipids |
| 1592 | Carbon particles (source of particles unknown) |
| 1653 | Amide I (symmetric C=O stretching mode of proteins) and C=C lipid stretch (Lipids and proteins) |

**Table ST2.** Confusion matrix for PLS-DA derived classification model showing correct classification rates (%) averaged over 1000 iterations.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | | **Predicted Class Labels** | | |
|  |  | **Control** | **MCL** | **MDL** |
| **Reference Labels** | **Control** | 90.1 | 0.1 | 9.8 |
| **MCL** | 1.0 | 97.7 | 1.3 |
| **MDL** | 20.9 | 0.7 | 78.4 |

**Table ST3.** Correct classification rates (%) of the PLS-DA-derived model using leave-one-mouse-out protocol (MD and MC refer to mouse models with MDA-MB-231 and MCF-7 tumor xenografts, respectively) with background subtracted spectra.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Mouse excluded** | **Correct classification rate (%)** | | | **Chauvenet’s criterion for MDL**  (n = 10; τmax = 1.96) | |
| **Control** | **MCL** | **MDL** | **τ = |xi - xmean|/σ** | **Result** |
| None | 95.4 | 95.6 | 75.1 | 0.71 | Retain |
| MD #1 | 85.7 | 94.2 | 81.5 | 0.04 | Retain |
| MD #2 | 90.1 | 94.8 | 76.8 | 0.52 | Retain |
| MD #3 | 100.0 | 96.0 | 100.0 | 2.18 | Eliminate |
| MC #1 | 88.5 | 96.3 | 83.0 | 0.21 | Retain |
| MC #2 | 96.3 | 95.5 | 74.5 | 0.77 | Retain |
| MC #3 | 96.2 | 96.5 | 73.1 | 0.95 | Retain |
| Control #1 | 96.7 | 93.4 | 73.7 | 0.87 | Retain |
| Control #2 | 98.0 | 94.0 | 84.8 | 0.42 | Retain |
| Control #3 | 95.6 | 94.1 | 89.6 | 0.97 | Retain |



**Figure S1.** Radial visualization plot showing clusters formed by spectra recorded from lung samples of sacrificed mice bearing MDA-MB-231 and MCF-7 breast cancer xenografts as well as controls without xenografts after autofluorescence background subtraction.