Correlated Mass Spectrometry Imaging and Confocal Raman Microscopy for Studies of Three-Dimensional Cell Culture Sections

Dorothy R. Ahlf\textsuperscript{1,2}, Rachel N. Masyuko\textsuperscript{1}, Amanda B. Hummon\textsuperscript{1,2*}, and Paul W. Bohn\textsuperscript{1,3*}

\textsuperscript{1}Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556
\textsuperscript{2}Harper Cancer Research Institute, University of Notre Dame, Notre Dame, IN 46556
\textsuperscript{3}Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, IN 46556

**Supplemental Information**

![Graph](image)

**Figure S1.** Expanded CRM spectra 2700-3200 cm\(^{-1}\) from different regions, the necrotic core/center region, and the proliferating edge/periphery.
**Figure S2.** (Left) High resolution CRM image (1600-1700 cm\(^{-1}\)) from the necrotic core showing small features consistent with cellular debris composed principally of protein. (Right) High resolution CRM image from the proliferating edge showing localized areas of high intensity in the same amide I band not found in the necrotic core.

**Figure S3.** Comparison of CRM image of the C-H stretching region, 2800-3200 cm\(^{-1}\) (left) and that from the protein region 1600-1700 cm\(^{-1}\) (right), shows the differences in spatial distribution of the two bands within one 50 \(\mu m\) region within the necrotic core.
Correlated Mass Spectrometry Imaging and Confocal Raman Microscopy

Figure S4. High molecular weight MALDI-MSI image showing putative protein peaks by spectra summed across the image (top) and the spatial distribution of two selected peaks at m/z 10103.1 and 9443.9 (bottom).

Control Tests for Correlated Imaging

In order to test the initial findings of this protocol, many control tests were performed, a few of which are described here. First, two spots of 1 µL of 5 pm ubiquitin and myoglobin were spotted adjacent to one another. The physical fiducial mask was added to the dried spots and the area was imaged using the full correlated imaging protocol. Principal components successfully identified and localized each biological molecule. This same method of spotting and adding the fiducial mask was performed with 5-fluorouracil, a small molecule in the metabolite range of interest biologically for its role as a chemotherapeutic, and ubiquitin. Though the MSI was performed only in the metabolite range, the CRM was able to localize the ubiquitin away from the 5-fluorouracil. Only the 5-fluorouracil signals were colocalized.
**Figure S5.** Principal components determined for the spheroid slice shown in Fig. S4. The first three principal components determined to be statistically significant for each method, MSI (left), and CRM (right), are shown. Here the PCA is performed on the low mass portion of the MSI data only.