

## QUANTITATIVE CHEMICAL ANALYSIS OF SUBCELLULAR DOMAINS WITH MULTI-ISOTOPE IMAGING MASS SPECTROMETRY (MIMS)

Martin Schwartz(maschwartz@virginia.edu)<sup>1</sup>, Claude Lechene<sup>2</sup>

<sup>1</sup>University of Virginia, Box 801394, Charlottesville, VA, 22908, USA

<sup>2</sup>Harvard Medical School and Brigham and Women's Hospital, 65 Landsdowne Street, Room 535, Cambridge, MA, 02139, USA

With MIMS analysis, we can estimate the relative composition of each single pixel-voxel with respect to the simultaneously detected masses. Thus, even without exposing the cells or tissues to isotopically labeled molecules, we can obtain a measure of the gross cellular composition at the level of microdomains that covers an area just a few pixels in size. Endothelial cells were cultured on silicon supports, chemically fixed, dried and analyzed with MIMS (primary Cs<sup>+</sup> ion beam, 16kV, 1pA). The mass images of the surface of the cells were recorded in parallel at mass <sup>12</sup>C<sup>-</sup>, <sup>12</sup>C<sup>14</sup>N<sup>-</sup> and <sup>16</sup>O<sup>-</sup>. In order to show high dynamic range ratio images and to de-emphasize values resulting from data with few counts, a method of displaying the data as a hue-saturation-intensity transform was developed. These transformed images allowed us to outline and quantitatively analyze regions of interest from the histological mass image. We were surprised to find domains which are regularly spaced -every few microns- along the external edge of the lamellipodia. These domains, two voxels large with a pixel size of 234 nm, contained five-fold more nitrogen than neighboring pixels, from which they were highly statistically different. They very likely represent focal adhesion domains or regions of anchorage of the cell to its support via adhesion proteins. In striking contrast, the very same pixel-voxels that were rich in nitrogen were very poor in oxygen, with a <sup>16</sup>O<sup>-</sup> signal that was up to six-fold smaller than the neighboring pixels. This may mean that at the site of cellular attachment on its support, there was a lesser amount of glycoproteins. Thus, using MIMS parallel mass imaging without isotopic supplementation we have found domains with relatively high nitrogen/low oxygen content that are regularly spaced at the edge of endothelial cells. Their size is at most 600 nm, and they can be differentiated with high statistical precision from neighboring voxels.

Supported in part by research resource grant 9 P41 EB001974-04