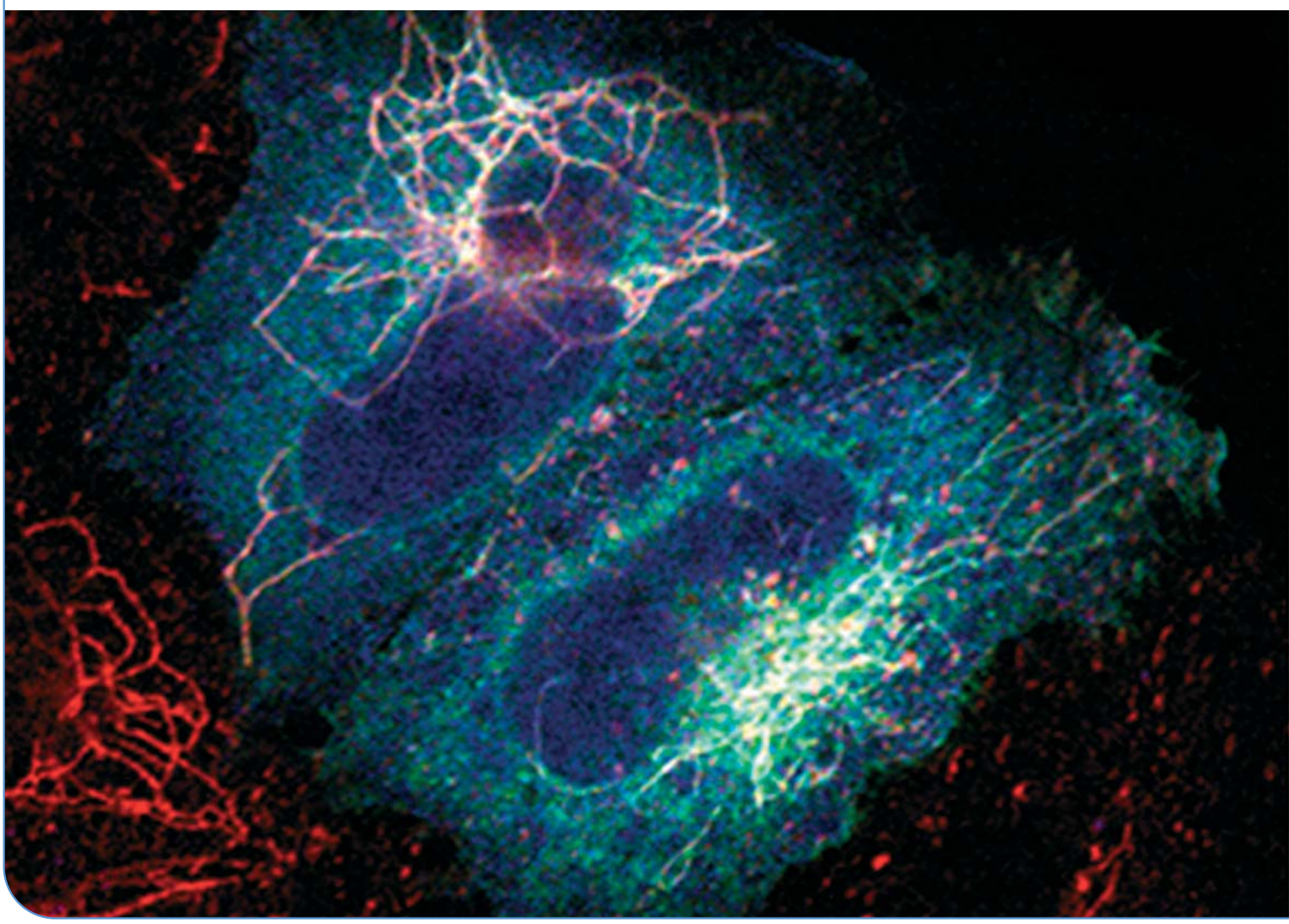
APPLICATION NOTE | Cells and Tissues



Reproduced with permission from Kail, M., Hollinshead, M., Kaufmann, M., Boettcher, J., Vaux, D. and Barnekow* A. (2005) Biology of the Cell Immediate online publication, © the Biochemical Society.

Molecular Ion Mapping of Duchenne Muscular Dystrophy Lipids

The ability of the TOF.SIMS 5 to image individual molecular compounds in order to obtain their detailed spatial arrangement was used to study the degenerative/regenerative processes in the muscles of a dystrophin-deficient model mouse. The specific distribution of different substances (fatty acids, vitamin E, triglycerides, phosphatidic acids, co-enzyme Q 9 and chlorine) were imaged from untreated mouse leg sections.

TOF.SIMS 5

Cells and Tissues

Analysis

In TOF-SIMS the molecules and atoms desorbed from the surface of the sample by bombardment with high energy primary ions are analysed by a time-of-flight (TOF) mass spectrometer. The molecules may be complete or fragments. By scanning the primary ion beam, maps of the distribution of the atoms and molecules are collected.
Recently sub-micron resolution molecular mapping has become a practical routine, principally because of technical improvements in the TOF.SIMS 5 by the addition of a Bismuth cluster primary ion source.

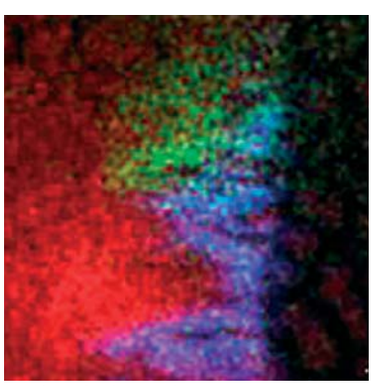
Sample Preparation

For extracted cells or tissue thin sections, it is clear that the less treatment, the more chance of retaining the original chemistry of the cell. For many imaging techniques some chemical preparation of the sample is required; for example in MALDI a matrix addition, and for fluorescence optical microscopy some fixation, staining and molecule labelling is needed. In TOF-SIMS fixation, staining, labelling and matrix, etc additions are not necessary because the instrument detects and analyses direct a wide molecular mass range and the untreated sample is used. However the analysis takes place in a high vacuum, so the samples must be freeze dried. Recent tests with pressure freeze drying and freeze fracturing have proved very successful.

Results

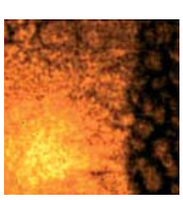
The images of individual substances show their distribution over the section. A concentration of chlorine is found in the destructured zone, known from other analyses to be regenerating. The chlorine is probably combined with sodium and potassium also found in this zone.
Fatty acids and triglycerides are located mainly in the blue intermediate zone, vitamin E, phosphatidic acids, and co-enzyme Q9 are found in both the blue and green intermediate zones, but there is much more vitamin E and coenzyme Q9 in the green zone than the blue, and it is suggested that these high accumulations mark oxidation stress and inflammatory reactions which can lead to muscle necrosis. Both zones are under oxidation stress and the green zone can be considered degenerative.
It was also found that the ratio of fatty acids palmitic acid to palmitoic acid, and the ratio of stearic acid to oleic acid varied between the zones.

The data was provided by Dr A. Brunelle, ICSN-CNRS, Gif-sur-Yvette, France.
The reference is: David Touboul, Alain Brunelle, Frédéric Halgand, Sabine De La Porte and Olivier Laprévote 2005, Journal of Lipid Research, Vol. 46, 1388-1395, July 2005

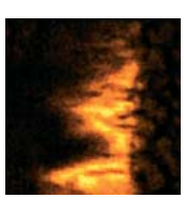


Overlay: Cl, Oleic Acid, Coenzyme Q9

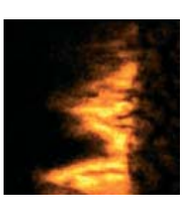
The 500 x 500 μm^2 overlay image of several ion maps of the mouse leg section shows an apparently healthy zone on the right (dark red/black), a destructured zone (red) on the left corresponding to that seen in the optical image, and two intermediate zones (green and blue)



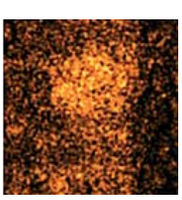
Cl



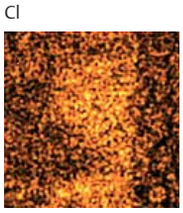
Palmitic Acid



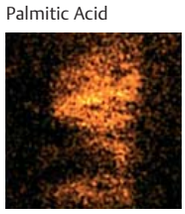
Oleic Acid



Vitamin E

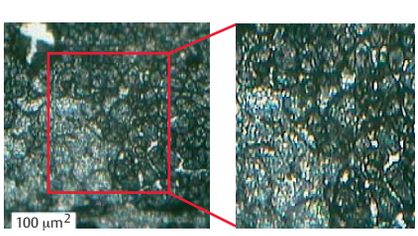


Phosphatidic Acids



Coenzyme Q9

Triglycerides



The optical image of the mouse leg section shows the area analysed by TOF-SIMS, enclosed by a red line. The destructured cells can be seen in the bottom left quarter of the area.

Heisenbergstraße 15 . 48149 Münster | Germany . Phone +49 (0)251 1622 100 . Fax +49 (0)251 1622 199 . sales@iontof.com . www.iontof.com