Mass spectrometric imaging, a novel way of visualising lipid distribution in tissue

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Visualisation of selected biomolecules in plant and animal tissue is routinely performed using immunohistochemistry and fluorescence microscopy. These techniques are very successfully used for protein imaging, however transgenic fluorescence tagging only works for gene products and immunohistochemistry relies on the availability of antibodies to the molecule under investigation. This is a limitation when trying to map the distribution of many smaller molecules including specific lipids.

Mass spectrometric imaging is an attractive alternative technique, as it relies on the molecular weight and sometimes fragmentation patterns of a molecule for identification and can therefore very specifically image individual lipid species based on direct physical measurements. Lipids are usually identified down to class and overall fatty acid composition, *i.e.* head group and total number of carbons and double bonds in the fatty acid constituents. Although it is possible to determine the precise makeup of the fatty acids by mass spectrometry, in an imaging context this is usually limited by system sensitivity.

A specific type of mass spectrometry imaging is matrix assisted laser desorption/ionisation (MALDI) imaging. In this technique the tissue surface is coated with a light energy absorbing matrix. A laser is used to desorb material from the sample in a raster pattern. The material desorbed at each spatial coordinate is analysed by a mass spectrometer providing spatially resolved mass and intensity information which can be transformed into an ion intensity map of the tissue.

One of the limitations of this technique compared to optical methods is spatial resolution. Until very recently instrumental limitations meant that most MALDI imaging work was performed at step-sizes of 50 μm. Imaging glucosylceramide in spleen tissue from a mouse model of Gaucher disease using an oversampling technique we demonstrate that step-sizes of 15 μm are achievable on an older generation mass spectrometer.

Another challenge in MALDI imaging is dealing with sample complexity. In conventional mass spectrometry many options are open to the analyst to fractionate samples prior to analysis, for example gas or liquid chromatography. In direct tissue analysis these options are limited by the way samples are introduced into the analytical instrument. We have pioneered the use of a commercially available novel separation technique, ion mobility separation, in MS imaging. This separation occurs *post* ionisation and can therefore be used in direct tissue analysis. Using this approach it was possible to separate the anti-cancer agent vinblastine from a near isobaric phosphatidylcholine species in the analysis of a whole-body section of a drug dosed rat.

In recent work we have been comparing tissue types found in prostate cancer biopsies. In these samples stroma can clearly be differentiated from tumour tissue based on differences in lipid composition of the different tissue types.