Metabolomics of Intact Tissues: Discrimination Between Different Regions of Osteolytic Lesions in a Multiple Myeloma Patient using 1H-HR-MAS NMR spectra

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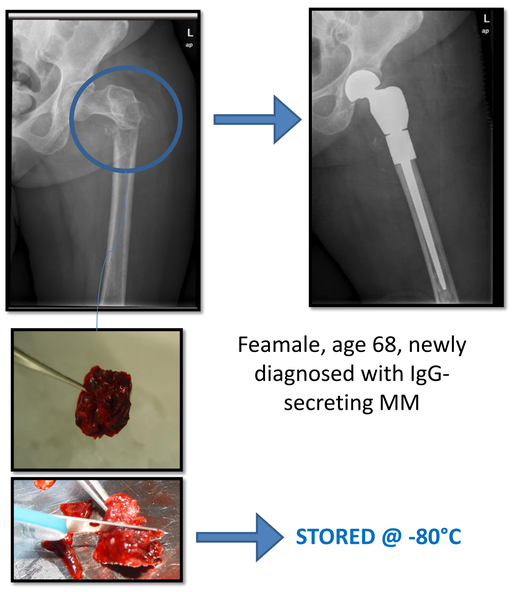
Here we present an integrated application based on the R-package MUMA and Mnova software for the processing, analysis and classification of different regions of osteolytic lesions in a MM patient's bone tissue biopsies.

The metabolic profiling or metabolomics of disease has proven useful to identify diagnostic and prognostic markers. Although the potential of metabolomics has been established in solid tumors (prostate, breast cancer and colon cancer), much less is known about its use in hematological malignancies or in the evaluation of bone lesions. We thus set out to develop the metabolomic study of myeloma-induced bone disease. To this aim, bone tissue biopsies have been collected from MM patient undergoing orthopedic surgery and analyzed by High Resolution – Magic Angle Spinning Nuclear Magnetic Resonance (HR-MAS NMR).

SAMPLING DURING PATIENT SURGERY

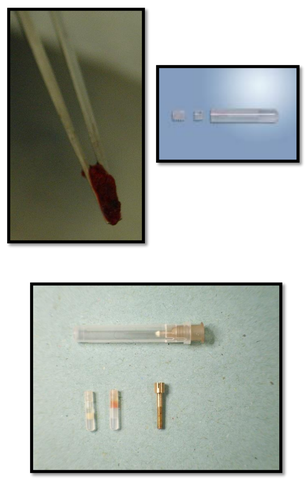


Feamale, age 68, newly diagnosed with IgG-secreting MM

STORED @ -80°C

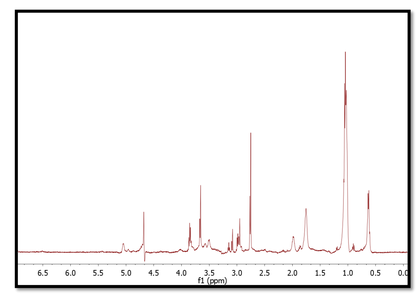
HR-MAS INSERT PREPARATION

FROM -80°C TO COLD ROOM

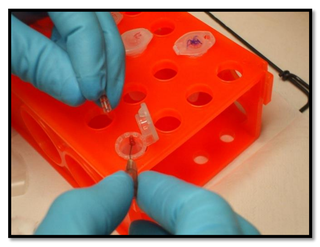


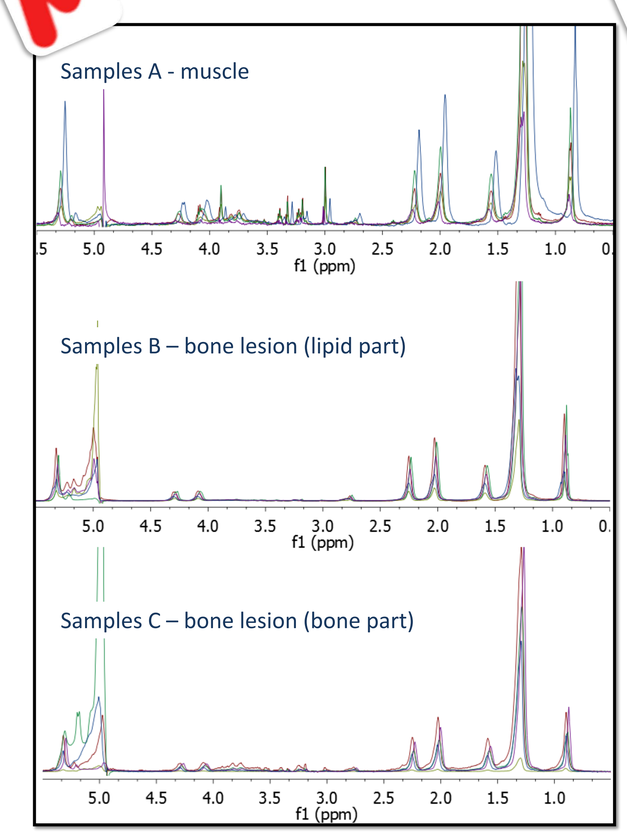
AGAIN STORED @ -80°C

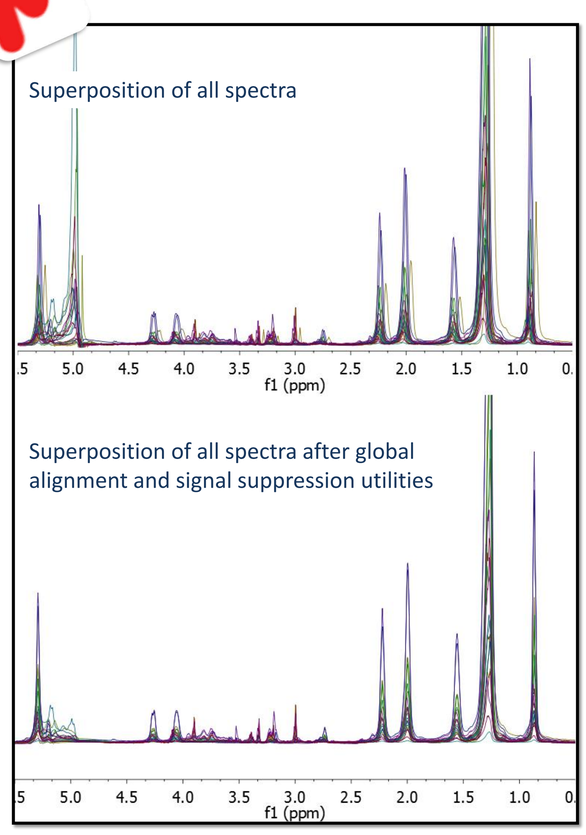
NMR SPECTRA ACQUISITION AND SAMPLE RECOVERY

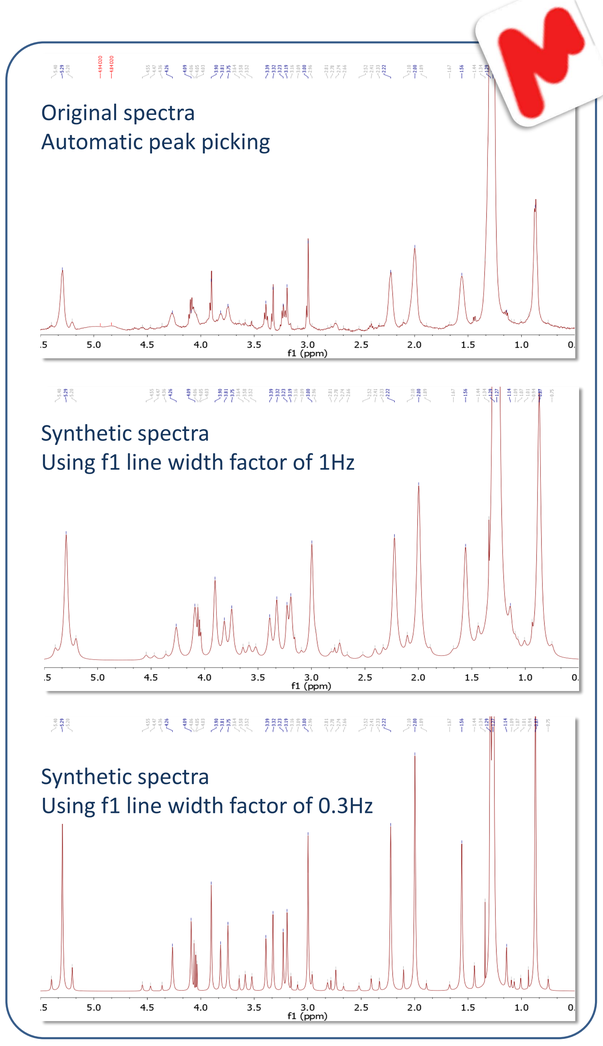


After approximately 20 minutes of experiment sample were remove from insert and place into formalin for further histopathological analysis







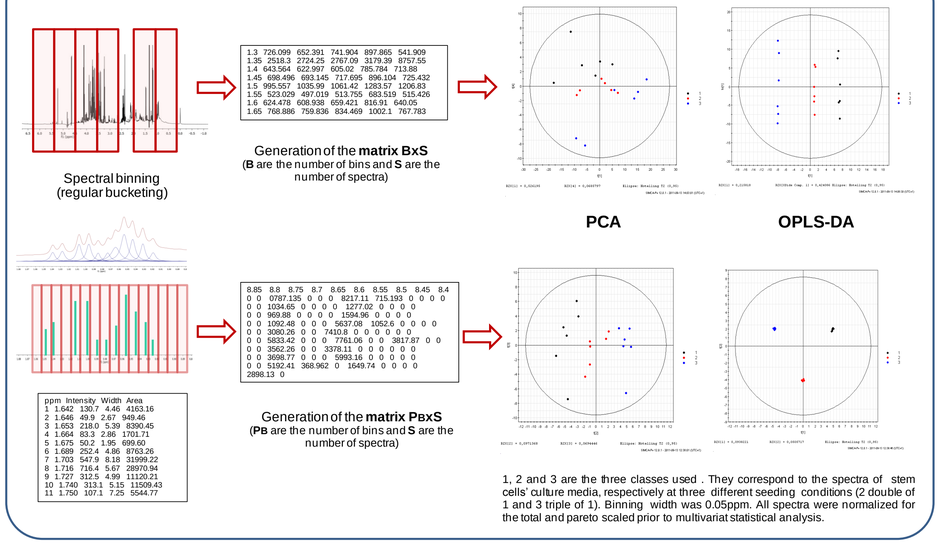


Since the actual nature of all the metabolites is rarely known in advance, metabolomics often uses alternative statistical evaluation methods, such as multivariate factor analysis. Such approaches require integration over predefined intervals (bins) and a meaningful integration of such intricate and artifact-burdened spectra may often be just as arduous as peaks fitting. Recently, a new algorithm called GSD (Global Spectrum Deconvolution) has been developed and made available in the Mnova software package (Mestrelab Research). GSD is capable of identifying even poorly resolved spectral signals and of fitting all recognizable peaks in even very complex 1D spectra. GSD produces a table of all detectable spectral peaks and their parameters. Such a table can be then used for various purposes like generation of artifact-free synthetic spectra as well as accurate binning.

Finally, both GSD-based binning matrix (BinMat) and standard binning were used as input to the in-house developed R-package MUMA (Multivariate & Univariate Metabolomic Analysis). MUMA will be soon available online for a free download. It performs total spectra normalization and scaling as well as both univariate and multivariate analysis.

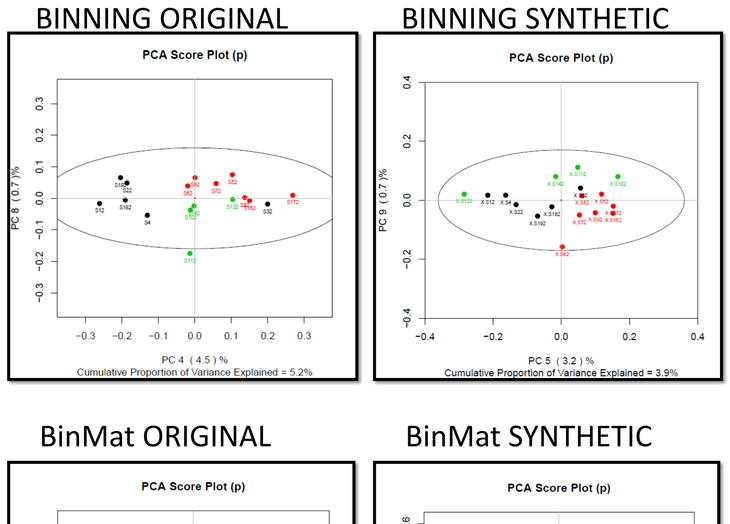
We compare here multivariate statistical analysis such as PCA and OPLS-DA on both original spectra and synthetic spectra, obtained under Mnova increasing line width with a factor of 1Hz. For both cases input matrixes for MUMA were obtained both using regular bucketing of 0.05ppm and by BinMat script with same bin width. BiMat is available under Mnova.

Spectral binning VS Peak list binning (BinMat)

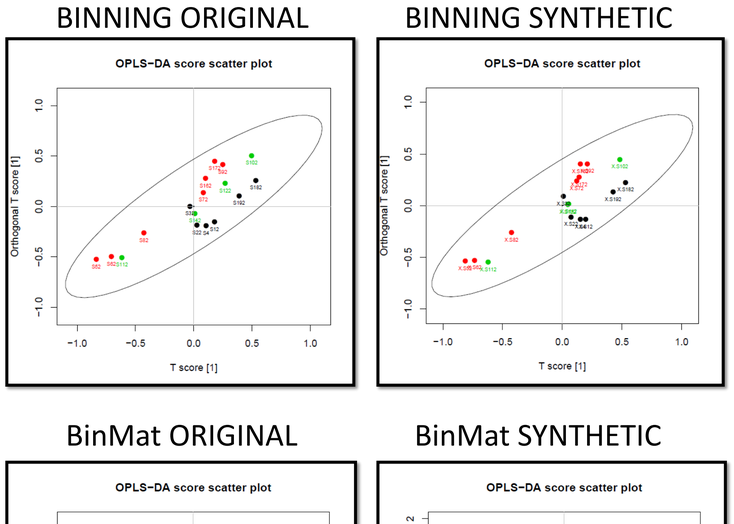


1, 2 and 3 are the three classes used . They correspond to the spectra of stem cells' culture media, respectively at three different seeding conditions (2 double of 1 and 3 triple of 1). Binning width was 0.05ppm. All spectra were normalized for the total and pareto scaled prior to multivariat statistical analysis.

PCA ANALYSIS



OPLS-DA ANALYSIS



Colors codes: A samples; B samples; C samples

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