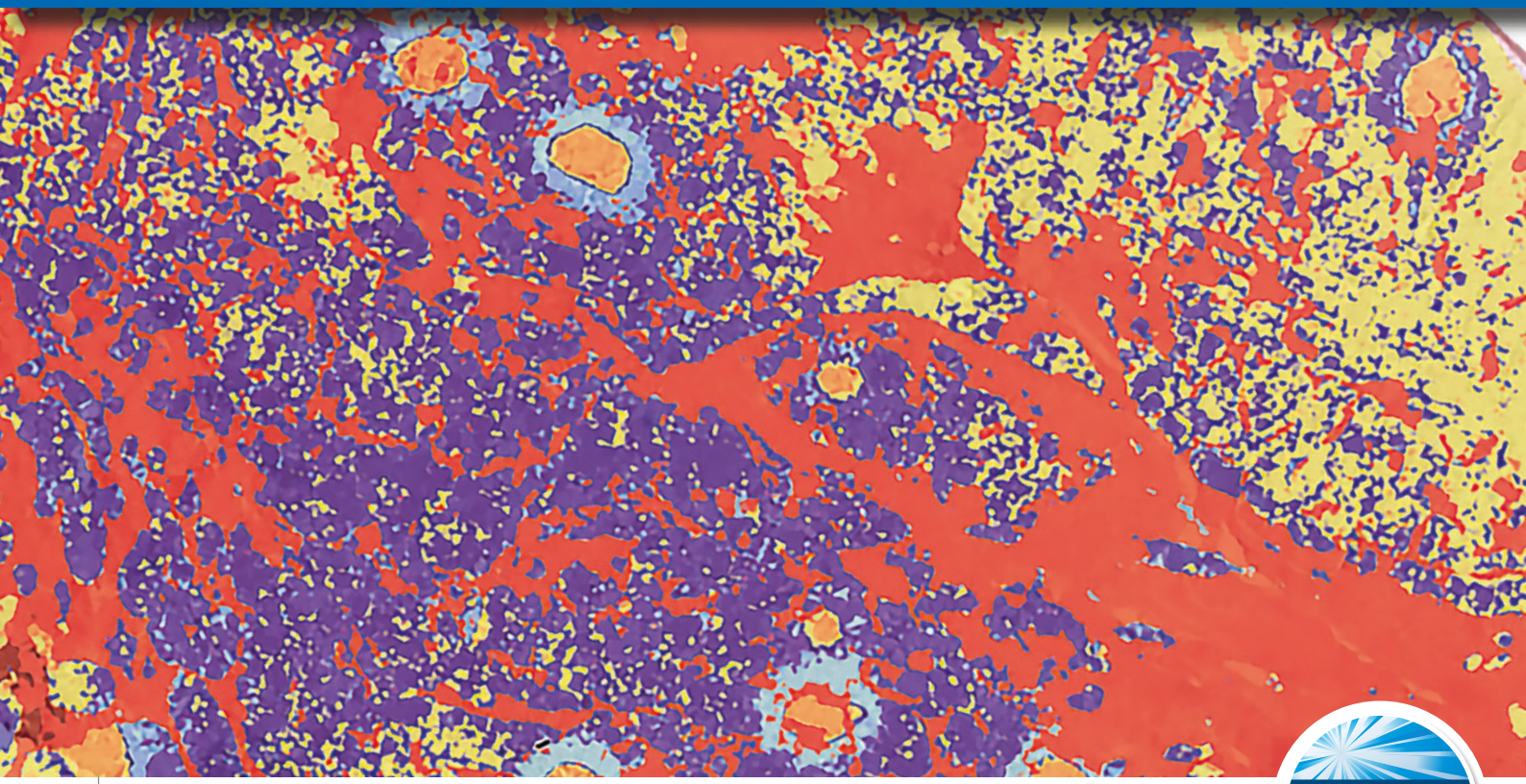

High Spatial Resolution Lipid Imaging

High Spatial Resolution Lipid Imaging Offers Promising Perspectives in Sustaining Diagnosis of Human Lymphoma.

Introduction

Human lymphomas represent a very heterogeneous group of tumors and their classification is not trivial. Besides histological examination, immunohistochemistry with several specific antibodies is essential for proper differential diagnostics. It was explored whether new MALDI imaging mass spectrometry methods with a high spatial resolution (10 µm) applied to the lipid mass range (400/620-1200 m/z) could provide relevant information for better identification of disease categories.

Keywords: resolution, speed, lipids, MALDI imaging, rapiflex MALDI Tissuetyper, SCiLSLab, lymphoma, tissue heterogeneity

Authors: Yousef El Aalamat [1,5], Arndt Asperger [2], Xian Mao [1,5], Thomas Tousseyn [3], Wim Waelput [4], Bart De Moor [1,5], Etienne Waelkens [5,6],
1. KU Leuven, ESA-SCD / iMinds Medical IT,Euven, Belgium; 2. Bruker Daltonik GmbH,Fahrenheitstrasse 4, 28359 Bremen, Germany; 3. Ku Leuven, Dept. Imaging and Pathology, Leuven, Belgium; 4. UZ-Brussel, Dept of Pathology, Brussel, Belgium; 5. KU Leuven, SyBioMa, Facility for Systems Biology based Mass spectrometry, Leuven, Belgium; 6. KU Leuven, Dept. of Cellular and Molecular Medicine, Leuven, Belgium.

Methods

Lipid Imaging: The performance of the high spatial resolution lipid imaging was demonstrated on four histologically-vaginated case studies of 5 µm thick sections of human lymph node. Cryosections were mounted on ITO glass slides and coated with 2.5-DHB matrix using sublimation. Imaging Mass spectrometry was done on a Bruker rapiflex MALDI Tissuetyper MALDI-TOF mass spectrometer. The acquired mass range extends from m/z 620 to 1200 (P1, P2) or from m/z 400 to 1200 (P3, P4), and the pixel grid had an interspot distance of 10 µm in both the x and y directions.

The acquisition speed was 32 pixels/sec with 200 lasershots/pixel. For each case study, an additional neighbouring slide (5µm) was prepared and stained with hematoxylin-eosin.

Histology: Histological examination was done on a neighbouring slide (5µm) stained with hematoxylin and eosin. Data processing: Initial data processing for spatial segmentation of the data was performed using the SCiLS Lab software. Standard TIC based normalization was performed and the mean MS patterns for each region were obtained using custom-developed methods.

Results

Figure 1: The lipid imaging demonstrated several clearly defined biomolecular patterns that correlated perfectly to the histological structure of the tissues. The high spatial resolution allowed zooming into anatomical structures of only a few cells, an important feature in case of the proper diagnosis of human lymphoma.

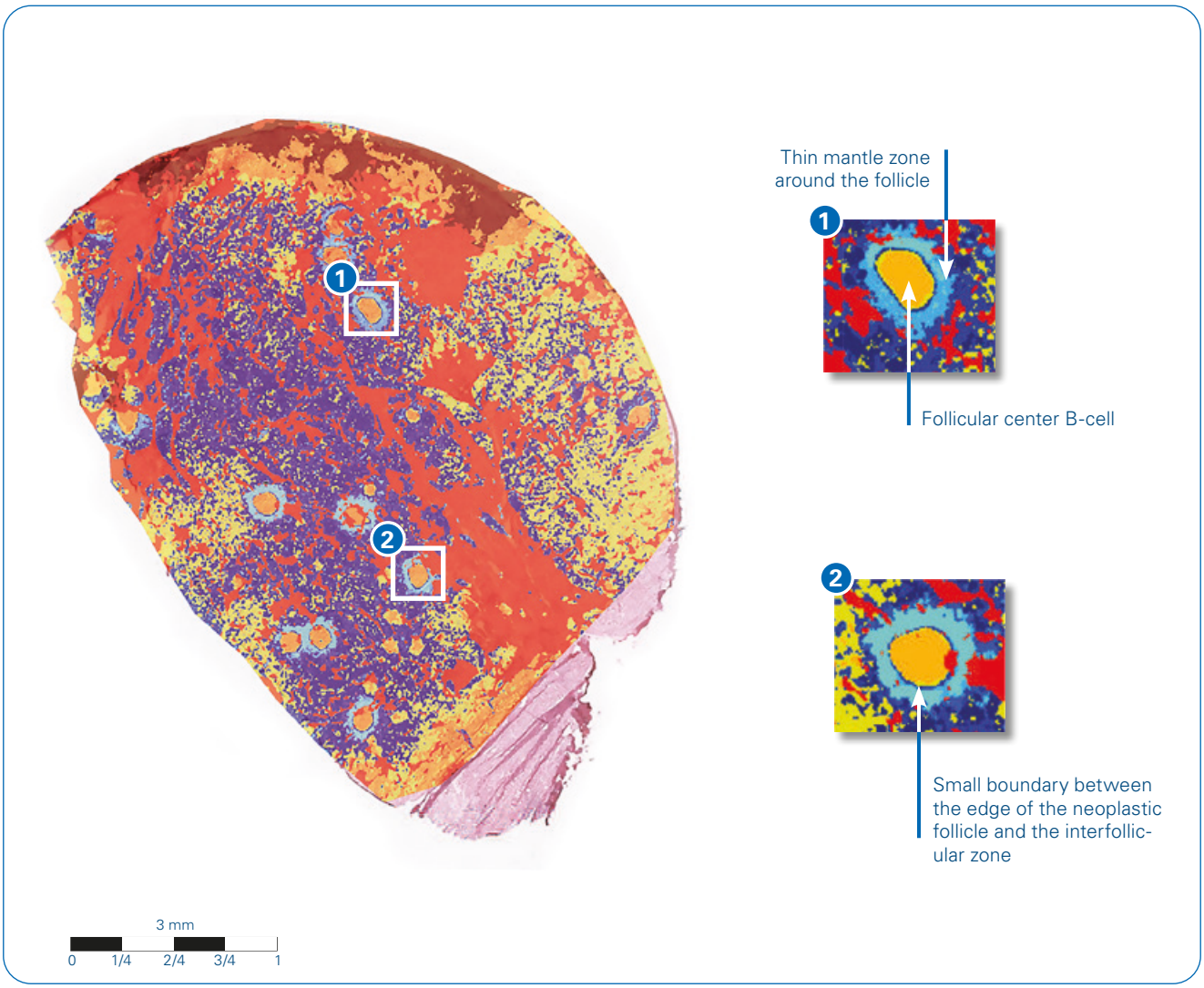

Figure 1: Overlayed H&E staining and Lipid Imaging MS

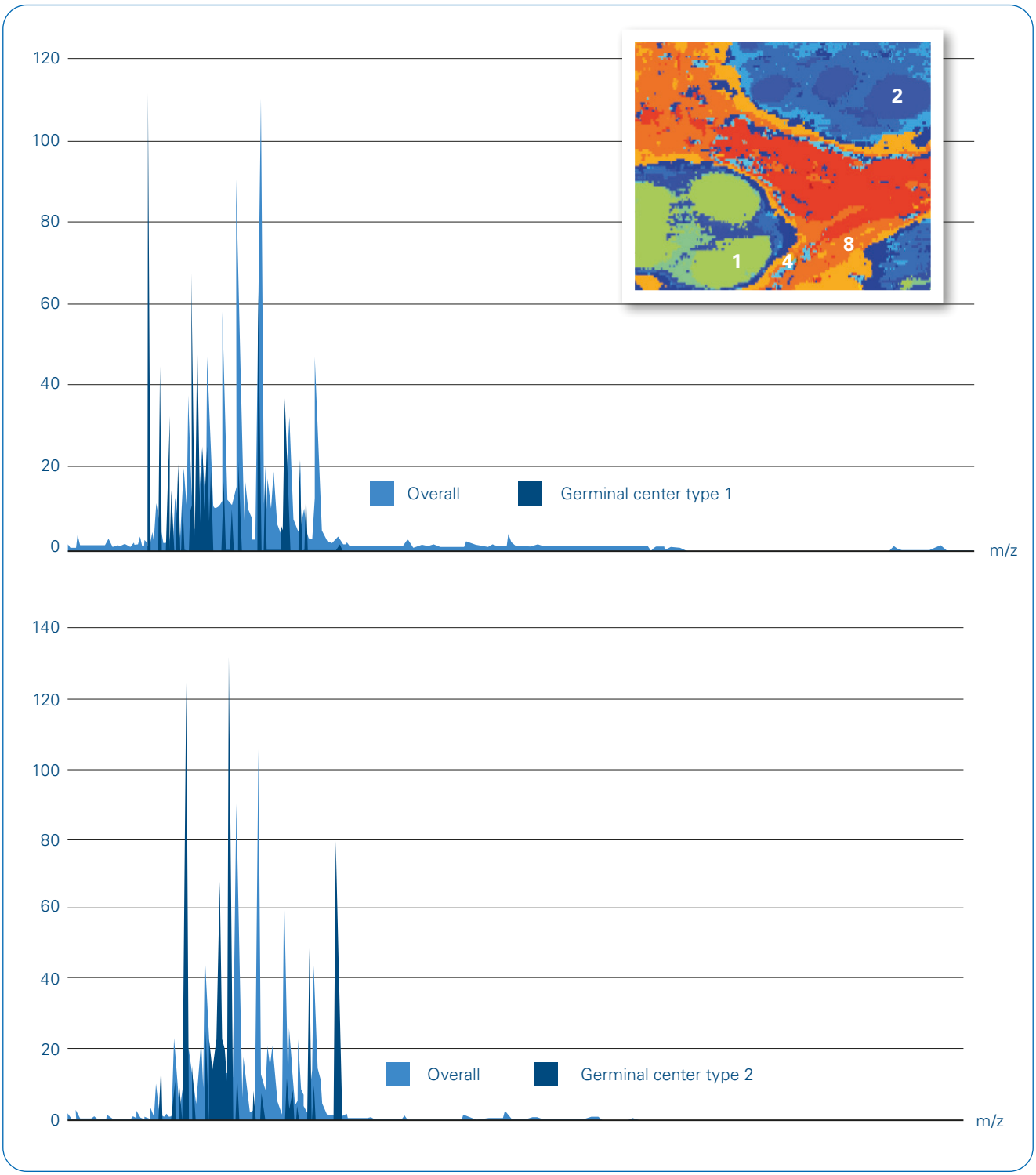

Figure 2: Histologically similar regions (Germinal center type 1 and 2) can yield significantly different lipid MS profiles

Figure 2: This fine "anatomical" resolution allows to dig in and extract MS-profiles from clusters of only a few cells. Histologically similar regions could show different mean MS profiles.

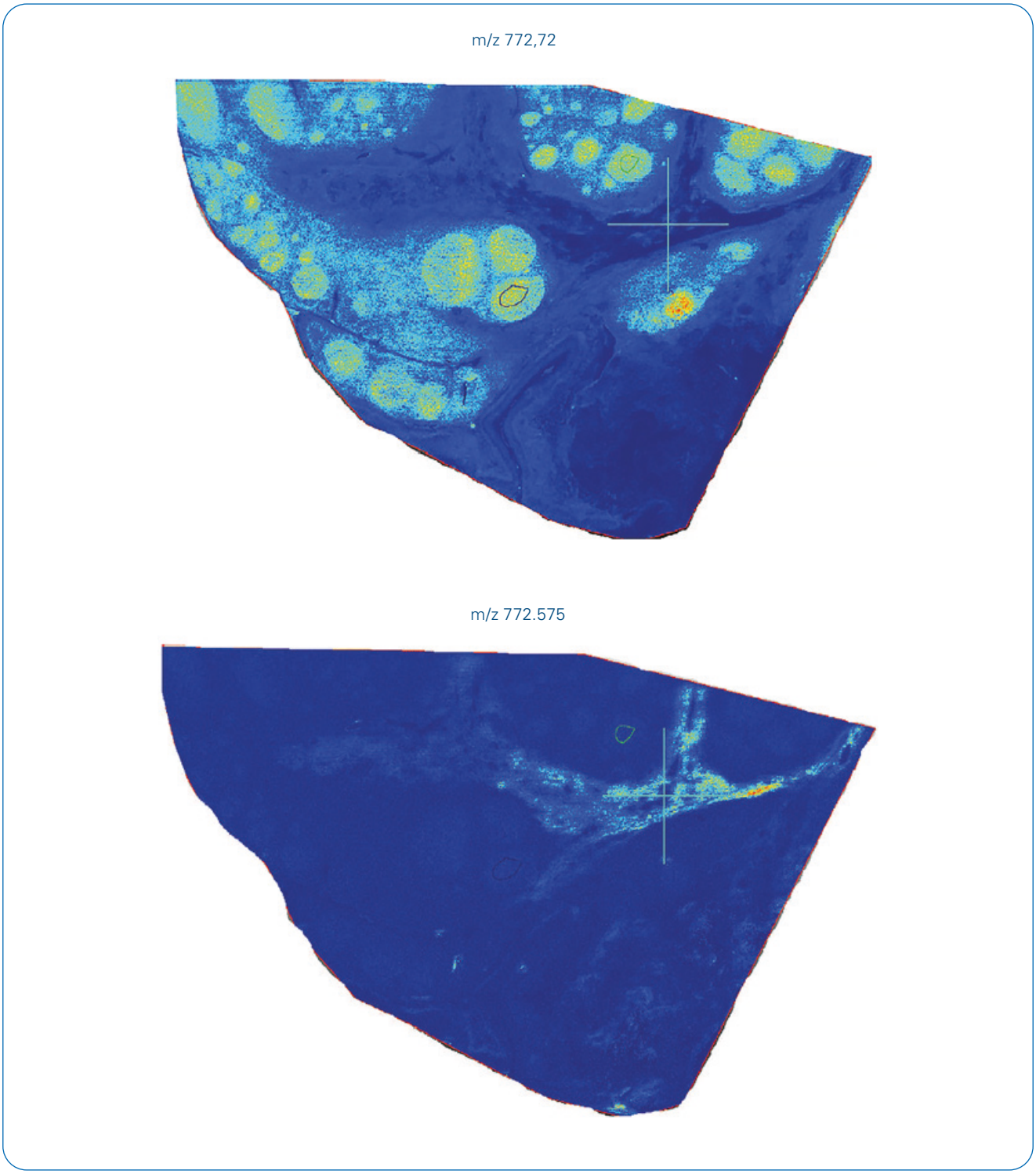

Figure 3: High mass resolution and mass stability achieved on the rapiflex instrument allow for imaging of nearly isobaric m/z features. The distance in mass between the two extracted m/z images shown here is only 0.14Da.

Figure 3: Not only high spatial resolution, but also high mass spectrometric resolution and mass stability is important to make lipid imaging a valuable addition or alternative to immunohistochemistry. A mass difference of only 0.15 Da can already produce distinct ion images (this is in agreement with Marien et al). The mass stability, as measured by comparison of average mass spectra generated from three different regions across the tissue section, was excellent across the full data acquisition sequence.

Figure 4: The highly multiplexed lipid imaging data showed that the lipid composition between various lymph node tissue regions and various lymphoma cases differs sufficiently to identify distinct cell populations. This data provides a new and previously unexplored information (lipid distribution !) helping proper classification.

Conclusions

- Low m/z range lipid mass spectrometry imaging, with preserving high spatial resolution allows to recognize the cell-specific distribution of lipids in tumours of lymphoid tissue.
- This new method has the promise to expand the existing histochemical, immunohistochemical and molecular arsenal to identify, diagnose and subclassify tumours of the lymphoid tissue.
- The robustness of the rapiflex instrument enabled the acquisition of the entire MALDI imaging dataset presented here, i.e. more than 2 million pixels (200 laser shots per pixel), at a consistantly high level of data quality without the need of intermediate cleaning of the MALDI ion source.

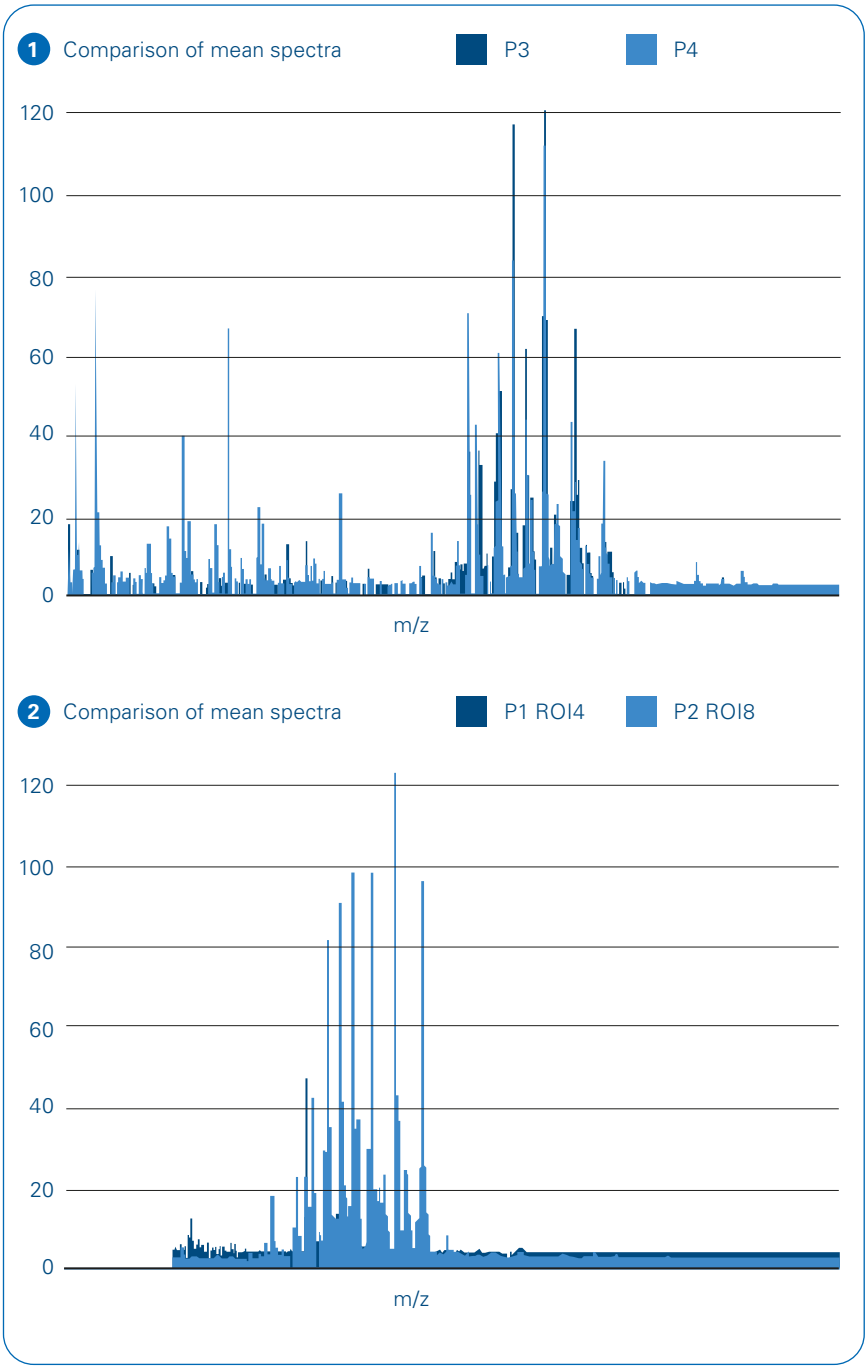

Figure 4: 1: Lipid patterns of 2 lymphoma cases (P3 & P4) with similar histological diagnosis
2: Lipid patterns of Non-germinal center lymphocytes from 2 lymphoma cases (P1 & P2)

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Bruker Daltonics GmbH & Co. KG

Bremen · Germany
Phone +49 (0)421-2205-0

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com - www.bruker.com