SCiLS Lab 2D: Comparative Analysis for Uncovering Discriminative M/z-markers

MALDI imaging mass spectrometry (MALDI-imaging) is a spatially-resolved bioanalytical technique detecting proteins, peptides and lipids directly from tissue. MALDI-imaging is a label-free, exploratory method and therefore a tool of choice for discovering clinically relevant biomarkers. In this application note, we used SCiLS Lab to analyze MALDI-imaging data from skeletal muscle sections[1] with the aim to find m/z-markers discriminating pathophysiological regions (trauma; trauma adjacent; healthy) in injured skeletal muscle.

DISCRIMINATIVE M/Z-SIGNALS

MALDI-imaging can help reveal candidates for disease biomarkers by comparing tissue samples corresponding to different conditions. This can be achieved by relating the spatial distribution of ions with the histological annotation of the tissue[2,3].

SCiLS Lab is an advanced software for visualization, analysis, and interpretation of MALDI-imaging data, capable of handling large individual datasets and whole sample cohorts with equal ease.

SCiLS Lab can be used to find m/z-signals discriminating different biological states and thus facilitate the discovery of novel biomarker candidates.

In this application note, we demonstrate how m/z-signals discriminating pathophysiological alterations of traumatized muscle tissue of rat can be easily detected.

METHOD

We performed the MALDI-imaging workflow on four sections from traumatized muscle (including trauma and trauma adjacent) and four sections from a healthy muscle sections of rat.

Sample preparation and MALDI-imaging data acquisition were carried out according to the protocol developed for formalin-fixed paraffin-embedded (FFPE) samples described in [1]. The MALDI-imaging dataset consisted of 39,926 spectra acquired with a raster width of 80 µm, each and a mass range of 800 to 3500 m/z.

Subsequently to MALDI measurements, the sample was stained with hematoxylin and eosin (H&E). Conventional histological analysis classified pathophysiological alterations to statuses trauma (tm), trauma adjacent (tam), and healthy (hm), see FIG. 1.

In FIG. 2, the m/z-image of m/z 1488 is shown which has high intensity in the tam and hm region.

For automatically finding masses discriminating traumatized (tm) and healthy muscle (hm and tam) in SCiLS Lab, we used the receiver operating characteristic (ROC) tool. It is a univariate measure quantifying how well a selected m/z-value discriminates two different states.

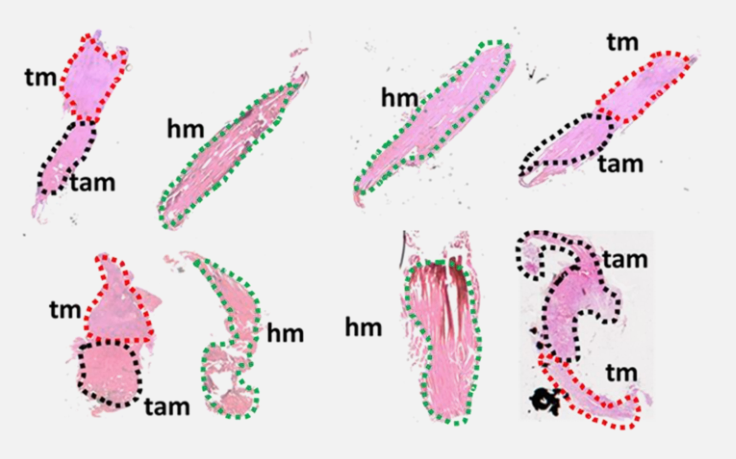

FIG. 1 H&E staining of the healthy (green) and the injured muscle (trauma in red, trauma adjacent in black) of rat.

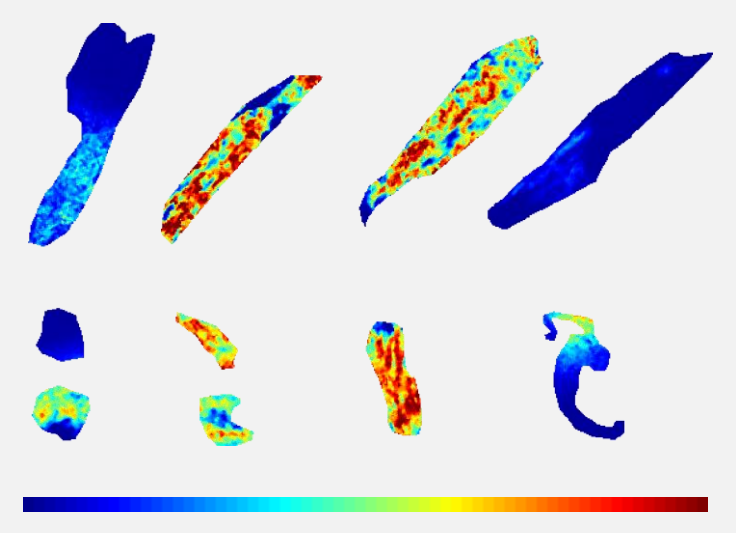

FIG. 2 Spatial distribution of m/z 1488±0.03% for the MALDI-imaging dataset of the sections shown above. Visualization was performed in SCiLS Lab with edge-preserving image denoising and automatic hotspot removal applied.

Calculation of a ROC for one m/z-value involves estimating sensitivity and specificity values for a trivial threshold classifier and then plotting a curve for the computed values. The area under the ROC curve (a.k.a. AUC value) assumes values between 0 and 1 and expresses the discrimination power of the m/z-signal in one value.

A perfect discrimination would yield an AUC value equal to 1 (abundant in group 1) or 0 (abundant in group 2). The closer the AUC to 0.5, the less useful the m/z-value, and the closer it is to 1.0 or 0.0, the more suitable the m/z-value is to be used as a univariate criterion[2].

COMPARATIVE ANALYSIS

Analyzing MALDI-imaging data of the muscle sections in SCiLS Lab, we found 108 m/z-values highly discriminating healthy (hm and tam) and the injured muscle (tm) having an AUC value larger than 0.85 or smaller than 0.15.

Before carrying out the ROC analysis, the spectra were preprocessed in SCiLS Lab by using baseline removal and TIC normalization during import of data. To find discriminative m/z-markers, all spectra from healthy (hm and tam) sections have been statistically compared with all spectra from injured (tm) section by means of ROC. In FIG. 3, two detected m/z-signals differentiating healthy and injured muscle are illustrated, FIG. 4 shows one of the corresponding ROC curves. Their AUC values are as follows:

Discriminating m/z-value	AUC value
m/z 1105.7 ± 0.03%	0.078
m/z 903.5 ± 0.03%	0.915

The list of m/z-markers can be easily exported for a subsequent analysis, for example, to provide the basis for their follow-up molecular identification.

KEYWORDS

MALDI imaging mass spectrometry, data analysis, muscle injuries, feature extraction, receiver operating characteristics (ROC)

SUMMARY

- In SCiLS Lab, spectra from different samples and/or patients can be grouped.
- Discriminative m/z-markers can be automatically found by means of receiver operating characteristic (ROC).
- SCiLS Lab can analyze all m/z-signals without restricting the search to a list of potential peaks.
- Candidate m/z-markers can be exported for subsequent analysis.

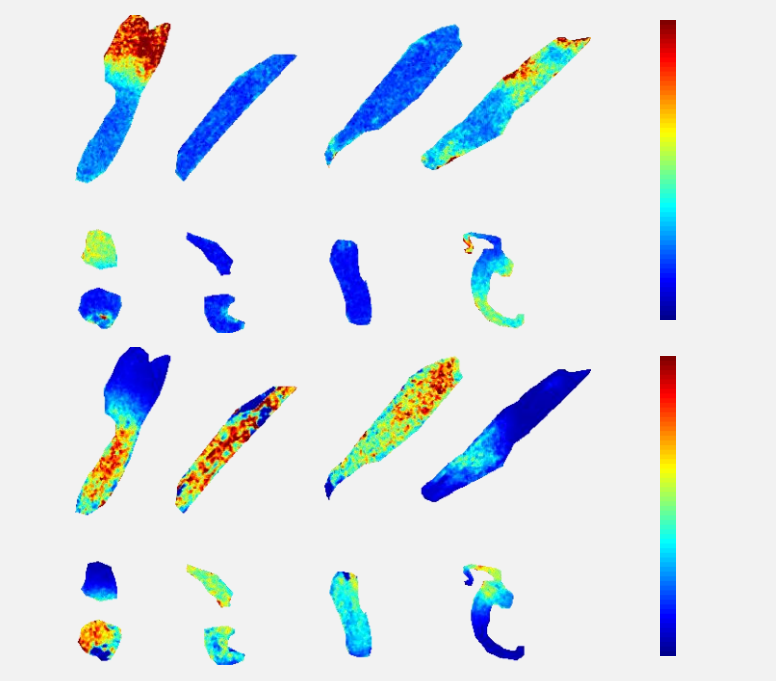

FIG. 3 Spatial distribution of ions discriminating healthy and injured muscle of the MALDI-imaging data. The m/z-value 1105.7±0.03% (AUC = 0.078) is abundant in the tm region, the m/z-value 903.5±0.03% (AUC = 0.915) is abundant in the hm and in the tam region. Visualization was performed in SCiLS Lab with edge-preserving image denoising and automatic hotspot removal applied.

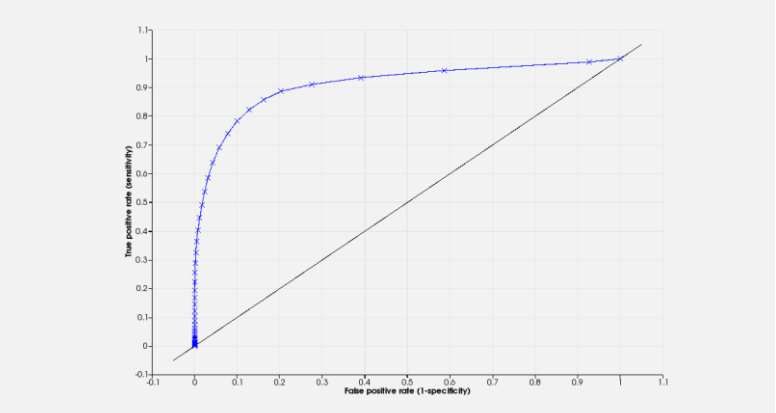

FIG. 4 The ROC curve of m/z-value 903.5±0.03% for healthy (hm and tam) versus the injured muscle (tm).

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