SCiLS Lab 2D: Discovery of m/z-markers co-localized with annotated regions

MALDI-imaging mass spectrometry, also called MALDI-imaging has proven its potential in proteomics and was successfully applied to various types of biomedical problems. In histopathology, MALDI-imaging is used as a tool to detect markers discriminating a particular tissue type or morphological structure. Typically, such structures are highlighted by an experienced histologist based on a visual inspection of histologically stained sections. Here, we show how the SCiLS Lab 2D software can be used to automatically elucidate m/z-markers exclusively co-localized with these annotations.

MANUAL ANNOTATION

Tissue samples subjected to MALDI-imaging samples can be stained post-acquisition using conventional histological methods, such as H&E staining. Using appropriate software, this staining can then be superimposed with the MALDI-imaging results. In particular, the virtual microscopy approach supported by Bruker Daltonik's flexImaging software (version 3 or higher) allow integration with true microscopic resolution images[1].

This allows for detailed annotation by experienced histologists (FIG. 1). Annotations can be imported from flexImaging or drawn directly in the SCiLS Lab 2D software, which then allows the elucidation of discriminative m/z-markers for each annotated feature.

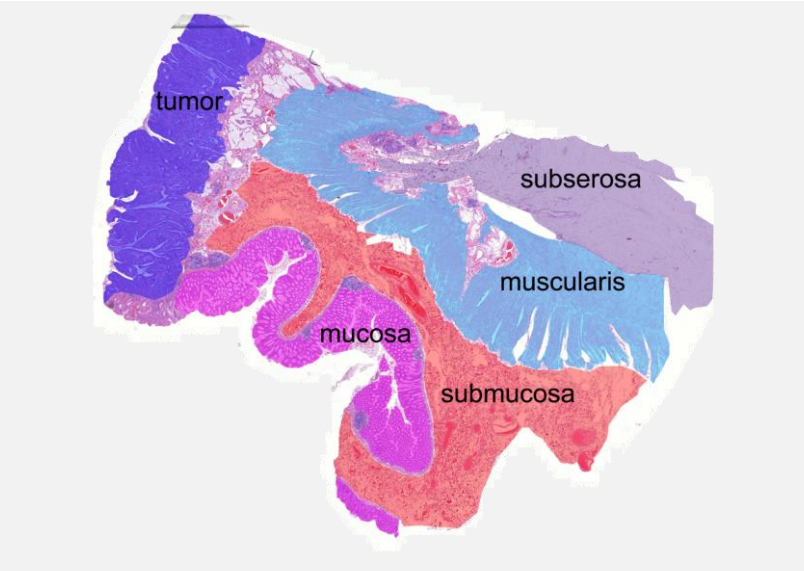

FIG. 1 Histological annotation of a human colon cancer sample imported from flexImaging into SCiLS Lab 2D.

METHOD

Finding molecular markers (i.e. m/z-values) specifically co-localized with annotated regions (i.e. having high intensities within the region and low intensities in the remaining sample area) is one of the most common requests for MALDI-imaging data analysis. For example, FIG. 2 shows the spatial distribution of m/z 2,089 which is co-localized with the *muscularis* region from the annotation in FIG.1.

Elucidation of co-localized masses is achieved in the SCiLS Lab 2D software by calculating the correlation of each m/z-image with a spatial mask derived from the annotation's shape and selecting strongly correlated m/z-values. In SCiLS Lab 2D, we use the Pearson correlation and consider only statistically significant correlations (usually with $p \leq 0.05$).

These co-localized m/z-values can be used to find m/z-values of compounds of high intensity in a specific region (e.g. tumor markers).

The annotation can be either imported from flexImaging, or drawn manually overlaid with the histologically-stained microscopy image, or created automatically by performing spatial segmentation.

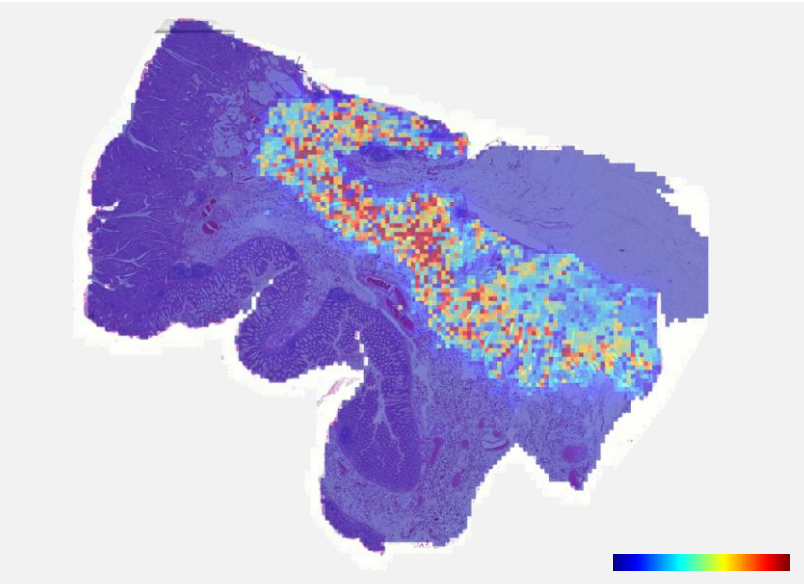

FIG. 2 Half-transparent overlay of the high-resolution microscopy image and m/z-value 2,089 which is co-localized with the *muscularis* region. Visualized in SCiLS Lab 2D with edge-preserving image denoising and automatic hotspot removal applied.

ANALYSIS OF COLON TUMOR DATA

Using SCiLS Lab 2D, we processed MALDI-imaging data of a human colon tissue section containing a tumor region. Tissue preparation and MALDI-imaging data acquisition were carried out according to standard protocols[2]. Altogether, 19,290 spectra were acquired with a lateral resolution of 150 µm, each spectrum covering a mass range of 600-4,000 m/z.

After MALDI-imaging analysis, the same section was H&E stained, scanned with a high-resolution slide scanner and co-registered with the MALDI-imaging data. MS-acquisition, co-registration, histological evaluation and annotation of MALDI-imaging data and H&E high-resolution image were all performed in flexImaging 3.0 and the results were imported into SCiLS Lab 2D.

For the tumor region, we have detected co-localized m/z-values by selecting a correlation threshold 0.4 ($p \leq 0.05$). Moreover, we computed the most strongly anti-correlated m/z-values for the tumor region (i.e. markers with a distribution anywhere except the tumor region).

FIG. 3 visualizes the spatial distribution of the three m/z-values most highly co-localized with the tumor region (m/z 1,629, m/z 1,591, m/z 945), and the three m/z-values with the highest anti-correlation values (m/z 1,106, m/z 837, m/z 1,139).

After the co-localized m/z-values were detected, in SCiLS Lab 2D each m/z-image can be overlaid with the microscopy image allowing a histologist to interpret the data, see FIG. 4. These results can also be conveniently exported for visualization into flexImaging. Co-localized m/z-values can be used as markers to classify a certain type of tissue or may represent the starting point for a downstream identification by established proteomic methods.

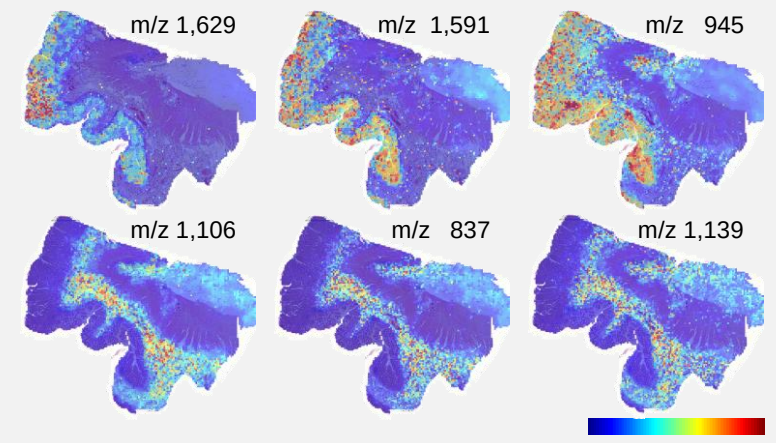

FIG. 3 Distribution of the three m/z values most highly co-localized with the tumor region and of the three m/z-values with the highest anti-correlation. Visualized in SCiLS Lab 2D with edge-preserving image denoising and automatic hot spot removal applied.

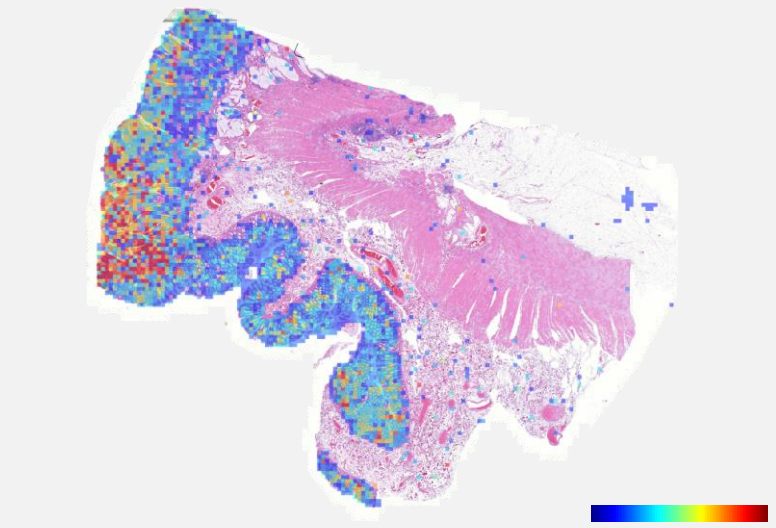

FIG. 4 The m/z-value 1,629 which is most highly co-localized with the tumor region (correlation 0.53). The MALDI-imaging data was co-registered with the microscopy image. Low intensities of the m/z-image are set transparent. Visualized in SCiLS Lab 2D with edge-preserving image denoising and automatic hot spot removal applied.

KEYWORDS

MALDI imaging mass spectrometry, colon tumor, data analysis, overlay of MALDI-imaging with microscopy, co-localized m/z markers

SUMMARY

- Co-registration of MALDI-imaging data and microscopy images is technologically possible.
- Prominent regions can be annotated in SCiLS Lab 2D or be imported.
- For specific regions, co-localized m/z-values can be calculated.
- m/z-images can be interpreted by superimposing them with high-resolution microscopy images.
- Co-localized m/z-values can be exported to flexImaging as result filters or to Microsoft Excel for subsequent analysis.

REFERENCES

1. Rauser et al. Approaching MALDI molecular imaging for clinical proteomic research: current state and fields of application. *Expert Rev. Proteomics* 2010, 7:927–41.
2. Alexandrov et al. Super-resolution segmentation of imaging mass spectrometry data: Solving the issue of low lateral resolution. *J. Proteomics* 2011, 75(1):237–245.

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