SCiLS Lab 2D: Quantitative Measure for Co-localization of m/z-images

MALDI imaging mass spectrometry is an analytical technique delivering information about the spatial distribution of molecules. MALDI-imaging is beneficial as a discovery tool, revealing molecules localized specifically to a particular feature or structure of the sample. The search for such molecules can be approached by identifying a template molecular ion with a distribution specific to the area of interest and then searching for other mass-over-charge (m/z) values co-localized with the template ion. With an application from photolithographic structuring[1], we show how the SCiLS Lab 2D software can be used to quantify co-localization of m/z-values in MALDI-imaging.

CO-LOCALIZATION

MALDI-imaging has recently emerged as an analytical technique of choice when carrying out a discovery study with the aim to reveal molecules of specific localization. The specification of the localization can be done by taking some known molecule as a template. FIG. 1 shows an UV-exposed negative photoresist layer, which is generally used to manufacture printed circuit boards. The m/z-values shown (469.25±0.05, top panel; 1,373.65±0.05, bottom panel) have high (m/z 469.25) or low (m/z 1,373.65) intensities along the conducting paths. With such template molecules the search for other co-localized molecules can be performed.

One way to find m/z-markers co-localized with m/z-values 469.25 and 1,373.65 is to navigate manually through all m/z-images to find all similarly localized m/z-values. Since a state-of-the-art MALDI-imaging dataset represents thousands of m/z-values, such a manual inspection is a time-consuming and error-prone endeavor[2]. With our software SCiLS Lab 2D, co-localized m/z-values can be discovered automatically.

METHOD

In SCiLS Lab 2D, a similarity measure for comparing the spatial distribution of two m/z-values is provided by calculating the Pearson correlation coefficient between their m/z-images[3].

The Pearson correlation coefficient quantifies the similarity between two m/z-images and takes values in the range from −1 to +1, where a value of +1 describes a perfect co-localization. A value of -1 characterizes a perfect anti-correlation meaning that the m/z-images have an opposite localization. A value of 0 implies that there is no correlation detected between the two considered m/z-images.

Given a certain m/z-value, the method can be applied for finding all m/z-markers with similar spatial localization. In SCiLS Lab 2D, the correlation can be calculated for any number of m/z-values.

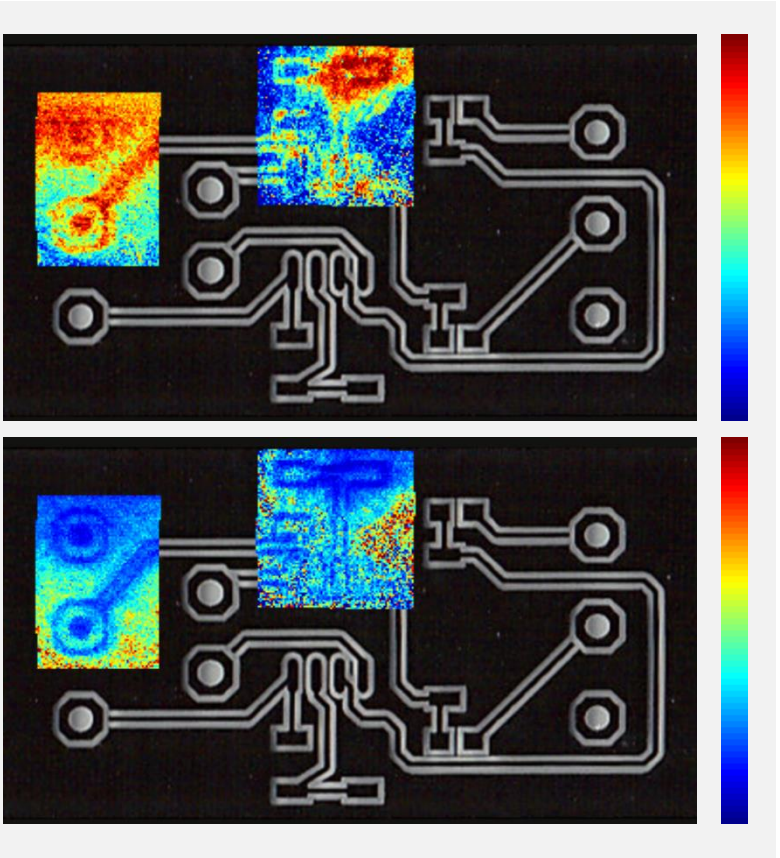

FIG. 1 Spatial distribution of m/z 469.25±0.05 and m/z 1,373.65±0.05 from a MALDI-imaging dataset of an UV-exposed negative photoresist layer. The m/z-value 469.25±0.05 has high intensities at the conducting paths (red) and the m/z-value 1,373.65±0.05 has low intensities at the conducting paths (green). Visualized in SCiLS Lab 2D with automatic hotspot removal applied.

ANALYSIS OF A PHOTORESIST LAYER

Using SCiLS Lab 2D, we measured co-localization of m/z-values in MALDI-imaging data of a photoresist layer. Surface preparation and MALDI-imaging data acquisition were carried out according to our protocol developed for polymer surfaces[1]. Altogether, about 13,200 spectra were acquired with a pixel size of 100 µm, each spectrum covering a m/z-value range of 100 to 4,500 m/z. Before calculating the co-localization, spectra were preprocessed in SCiLS Lab 2D by means of baseline-removal and TIC-normalization.

For the molecule to be used in the co-localization analysis we considered the novolac resin, more precisely, the undecamer of novolac resin with m/z-value 1,373.65 which has low intensity in the conductive paths, see FIG. 1. We have detected 30 m/z-values co-localized with m/z 1,373.65 with the correlation higher than a correlation threshold 0.45 ($p \leq 0.05$). FIG. 2 visualizes the spatial distribution of the four most co-localized m/z-values, which have the following correlation values:

Co-localized m/z-value	Correlation
m/z 233.1	0.55
m/z 1,055.4	0.54
m/z 1,692.9	0.54
m/z 471.2	0.53

The list of co-localized m/z-values can be exported to Microsoft Excel for a subsequent analysis.

Another possibility of illustrating the co-localization analysis is to sum up all co-localized m/z-values and to show them into one summary m/z-image. Such an image yields to a higher quality as compared to individual m/z-images since averaging of many m/z-images suppresses the noise. In FIG. 3, all co-localized m/z-values in the m/z-region from 730 to 2,500 are visualized. This corresponds to the repeating units of the novolac resin following from its synthesis[1].

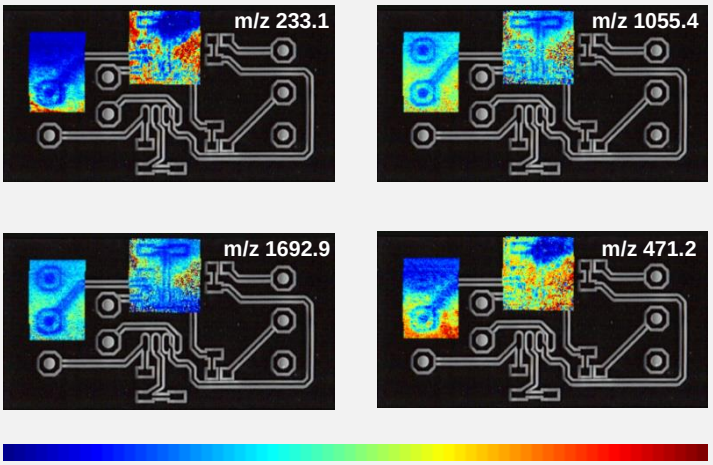

FIG. 2 Spatial distribution of the four m/z-values most co-localized with m/z 1,373.65. The m/z-images are ordered by decreasing correlation. Visualized in SCiLS Lab 2D with automatic hot spot removal applied.

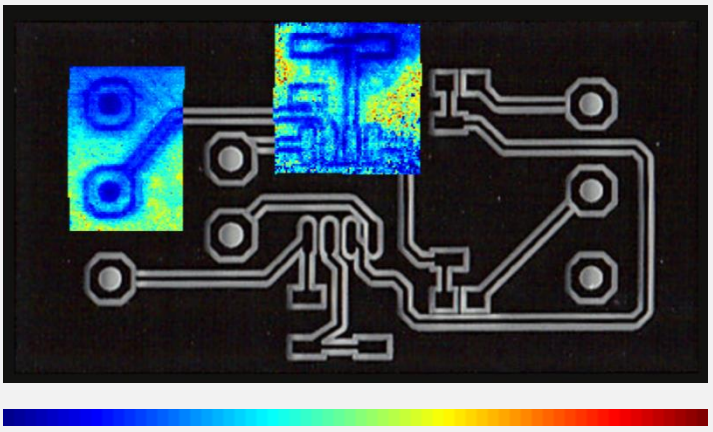

FIG. 3 Multivariate illustration of all co-localized m/z-values in the m/z-region from 730 to 2,500. Visualized in SCiLS Lab 2D with automatic hot spot removal applied.

SUMMARY

- For a template m/z-value, co-localized m/z-values can be calculated.
- Co-localization is quantified by calculating the Pearson correlation between two m/z-images.
- An efficient implementation in SCiLS Lab 2D allows one to any number of m/z-images.
- Co-localized m/z-values can be exported to either flexImaging or Microsoft Excel for subsequent analysis.

KEYWORDS

MALDI imaging mass spectrometry, data analysis, co-localized m/z-values, photolithographic structuring, circuit boards

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