ScatterJ: An ImageJ plugin for the evaluation of analytical microscopy datasets

F. ZEITVOGEL, G. SCHMID, L. HAO, P. INGINO & M. OBST
Environmental Analytical Microscopy, Center for Applied Geosciences, University of Tuebingen, Tuebingen, Germany

Key words. Analytical microscopy, data evaluation, ImageJ plugin, two-dimensional histogram, scatterplot, statistics.

Summary

We present ScatterJ, an ImageJ plugin that allows for extracting qualitative as well as quantitative information from analytical microscopy datasets. A large variety of analytical microscopy methods are used to obtain spatially resolved chemical information. The resulting datasets are often large and complex, and can contain information that is not obvious or directly accessible. ScatterJ extends and complements existing methods to extract information on correlation and colocalization from pairs of species-specific or element-specific maps. We demonstrate the possibilities to extract information using example datasets from biogeochemical studies, although the plugin is not restricted to this type of research. The information that we could extract from our existing data helped to further our understanding of biogeochemical processes such as mineral formation or heavy metal sorption. ScatterJ can be used for a variety of different two-dimensional (2D) and three-dimensional (3D) datasets such as energy-dispersive X-ray spectroscopy maps, 3D confocal laser scanning microscopy maps, and 2D scanning transmission X-ray microscopy maps.

Introduction

Spatially resolved analysis of analytical microscopy data can further our understanding of spatially heterogeneous processes that take place in complex systems. For example, many environmental sorption and transformation processes are known to exist on a bulk scale, although the underlying mechanisms are still not precisely understood. Environmental systems like soils or sediments are highly heterogeneous systems that vary on different spatial scales, which should be considered when trying to explain bulk effects. The development of spatially resolved analytical approaches that yield element-specific or chemical-specific (quantitative) maps was a milestone for better understanding such processes. However, although these maps are demonstrative, spatially resolved analytical data can contain information that is not obvious and as a result is often overlooked in the interpretation. In this paper, we demonstrate how analytical microscopy techniques in combination with a new software tool can provide deeper insights into environmental processes.

Spatially resolved analytical microscopy techniques are used for chemical analysis on the submicrometre scale in various scientific fields. These techniques include, e.g. energy-dispersive X-ray spectroscopy (EDX) mapping, scanning transmission X-ray microscopy (STXM), energy-selective imaging in transmission electron microscopy, high-resolution secondary ion mass spectrometry (NanoSIMS) and confocal laser scanning microscopy (CLSM). All are well documented in the literature (Williams & Carter, 1996; Goldstein et al., 2003; Bluhm et al., 2006; Neu et al., 2010; Behrens et al., 2012). Resulting datasets are commonly stored in the form of images (2D case) or stacks of images corresponding to parallel slices through a given volume (3D case). In these images, the grey value of a pixel (or voxel) represents a measure for the local concentration of a certain chemical species. Information on different chemical species is stored in separate grey scale images or in different channels of one multicolour image. Mapping analysis can focus on obtaining spatially highly resolved chemical information on a comparably small sample area, or on obtaining information on a larger sample area at intermediate spatial resolution.

This type of analysis can result in large datasets (typically $10^5$ to $10^7$ data points). Retrieving information from such datasets is not always straightforward. Although the data can contain quantitative or semiquantitative information, visual interpretation of image data can only be qualitative. In addition, depending on the nature of the samples as well as the analytical methods themselves, the quality of analytical microscopy datasets is not necessarily good in a statistical sense. The measurement error in the signal of single data points can be considerable, e.g. for methods with low count
rates, in particular when dealing with trace concentrations of the chemical species. Despite these challenges, analytical microscopy datasets consist of a large number of individual point measurements on a regular spatial grid, providing interlinked chemical and spatial information, and thus contain an abundance of statistical and spatial information that may not be perceptible at first sight. With our plugin, we provide a tool that helps to access information in analytical microscopy datasets that is not immediately obvious.

Several ways exist to evaluate such data; the most important ones are summarized in the following section.

One of the most common ways to evaluate large datasets is the calculation of statistical indicators. Frequently used are the Manders’ coefficients or Pearson’s coefficient that can be calculated as a measure of colocalization or correlation of two variables (Manders et al., 1993; Bolte & Cordelieres, 2006; Nakamura et al., 2007; Macdonald & Dunn, 2013). Linear relationships between two variables can be characterized using linear regression. Such calculations result in a small set of comparable indicators. However, reducing large datasets to a small set of numbers sometimes disregards original information, and can even lead to misinterpretation, e.g. if underlying assumptions are not met. For example, different methods of linear regression exist that are based on different assumptions on the error of the input variables (Rayner, 1985; Sokal & Rohlf, 1995).

Frequently, datasets consisting of different channels are presented by overlaying the respective images using different colours. Interpretation, however, relies solely on visual impression. A common approach to circumvent this problem is to generate a binary map of pixels for which the signal intensities meet certain criteria of correlation of colocalization. For this, several methods exist that apply different criteria, e.g. ‘colocalization maps’ based on thresholds and ratios (Bourdoncle, 2003) or automatic calculation of thresholds based on statistical indicators (Costes et al., 2004). Other procedures detect pixels with a similar chemical composition either through manual selection (Bright & Newbury, 1991; Bonnet, 2000) or using automated cluster analysis (Bonnet et al., 2002; Lerotic et al., 2004; Cutrona et al., 2005; Ward et al., 2013).

An approach that extracts spatially resolved information is the method of calculating ‘correlation maps’ implemented in the Image CorrelationJ plugin (Chinga & Syverud, 2007), which visualizes, for each pixel, the deviation from an ordinary least squares regression line.

A way to visualize the relationship of two variables upon each other over the whole dataset is to generate scatterplots or so-called 2D histograms. Scatterplots are frequently used in analytical microscopy (Bolte & Cordelieres, 2006; French et al., 2008; Miot et al., 2009; Obst et al., 2009). This is in fact the fundamental feature of our tool and will be described more thoroughly in the following methodology section.

To illustrate the use and functions of our plugin, we use three different types of datasets that were produced using different techniques as part of different experiments from current research on bacterial bioinmineralization and contain mostly information on the association of bacterial cells with iron minerals and heavy metals. Information on the experimental background is provided in the Supporting Information.

Materials and methods

Description of the plugin

Here we present a software tool that helps to statistically evaluate analytical microscopy datasets. The tool, which we called ScatterJ, was written in Java and designed as a plugin for the widely used, open-source, platform-independent image processor ImageJ (Abràmoff et al., 2004). Therefore, it can be used in an environment wherein a great variety of image processing functions is already provided. The plugin is published under the GNU General Public Licence (version 3) and can be downloaded at http://download.savannah.gnu.org/releases/scatterj/. Its main functions and advantages will be briefly described in the following. Further documentation is included in the plugin itself.

Input data

As input, the plugin requires a pair of images that represent the spatial distribution of two components (e.g. chemical species) within the same sample area. It is possible to use 3D data in the form of image stacks. The input images need to be of the same spatial origin, of the same dimensions, and of 8-bit grey scale type.

Generation of a scatterplot

The plugin extracts the grey values of two images (i.e. maps) of the same spatial region. For each pair of corresponding pixels, a data point is generated from their grey values. This results in a set of n data points (x,y), n being the number of pixels in each image and x and y being discrete values between 0 and 255. The result is displayed as a scatterplot, i.e. as a 256 × 256 matrix in which the element (x,y) contains the number of data points with coordinates (x,y). The numbers are represented on a colour scale. ScatterJ permits to adjust the display parameters such as axis limits, pixel size, and the colour scale in order to improve visualization of features of interest. More importantly, once the scatterplot is generated, ScatterJ offers several types of interactive analyses.

Backmapping

The backmapping function retraces an area in the scatterplot to the spatial domain of the respective input images. To do so, the user selects a region of interest (ROI) in the scatterplot using one of the ROI selection tools provided by ImageJ. ScatterJ detects those pixels in the original images that contribute to the backmapping function.

© 2014 The Authors
Journal of Microscopy © 2014 Royal Microscopical Society, 261, 148–156
to the selected area in the scatterplot. These pixels are highlighted in a map. Although it is already implemented in other software (Laumonerie & Mutterer, 2004; Pasquet & Bonnet, 2005), we decided to include it in our plugin for convenience and because interesting regions in the scatterplot might be better recognized using the improved, user-defined displaying options.

### Statistical calculations

The focus of the statistical methods included in ScatterJ is on linear regression. Three different regression methods are provided, i.e. ordinary least squares, major axis (MA), and reduced major axis regression. Ordinary least squares considers only one variable (i.e. the y-value) to be affected by measurement errors and thus is not much suited or even inappropriate for describing analytical microscopy data. Reduced major axis and MA acknowledge error for both variables, which leads to more suitable results (Sokal & Rohlf, 1995). In addition to showing the results of calculations such as the parameters of the regression lines and the root-mean-square-error as a measure of goodness of the fit, the regression lines can be drawn into the scatterplot. Calculations are based on Rayner (1985), Sokal and Rohlf (1995), Müller et al. (1979), Jackson (1980), and Jackson (1991). More information, including the mathematical equations, is provided in the Supporting Information.

### Deviation maps

The function ‘deviation maps’ helps to visualize, for each pixel in the input images, information about the relative position of the corresponding data point in the scatterplot. For each pixel, the distance of the corresponding data point to a calculated or user-defined reference line in the scatterplot is calculated. Calculated distances are displayed in a map using a colour scale. This function can be used in several ways. On the one hand, the reference line can be chosen arbitrarily at a seemingly interesting position. A similar method was used by Miot et al. (2011) to illustrate the correlation of Fe and protein in STXM maps. By contrast, deviation maps can be used to visualize the deviation of data points from a priorly calculated regression line. We consider this a generalization and extension of the above-mentioned function offered by Image Correlation[ (Chinga & Syverud, 2007). In our tool, several ways of calculating the distance are provided. In analogy to the deviation minimized by the different regression methods (see Supporting Information), the distance can be calculated as vertical distance, perpendicular distance, and square root of the product of horizontal and vertical distance. In addition, the angular distance can be used, i.e. the angle enclosed by the position vector of a data point and the reference line.

### Further options

Analyses can be confined to a subset of the data points in the spatial domain. If only a part or a particular feature of the images is to be included, this part can be selected prior to generating the scatterplot by using the ROI selection tools implemented in ImageJ. Correspondingly, a subregion of the scatterplot can be defined by selecting an ROI in the scatterplot, or by adjusting the axis limits so that only a part of the scatterplot is displayed. In addition, it is possible to exclude data points with coordinates (0,0) from statistical calculations or deviation maps. In the case of quantitative data, it is further possible to provide calibration factors that, for each axis individually, convert 8-bit grey values into meaningful units. In that case, calculations can be done in meaningful units instead of using grey values.

### Output data

All images that are generated, i.e. scatterplots and maps, are shown in separate image windows. Results of calculations are displayed in text windows. In addition, the contents of the scatterplot matrix can be written to an ASCII file.

### Example datasets

The first example dataset shows the association of Fe and organic carbon (OC) in a sample taken from a culture of Acidovorax sp. strain BoFeN1, which is a nitrate-reducing bacterium that can induce Fe(III) mineral formation (Kappler et al., 2005; Klueglein & Kappler, 2013). The sample was dried on a Formvar-coated transmission electron microscopy grid. STXM data were acquired at the soft X-ray spectromicroscopy 10ID-1 beamline of the Canadian Light Source (Saskatoon, Canada) and quantified using xIms2000 (Hitchcock, 2014). Images were converted into linear absorbance scale in units of optical density (OD) according to the Beer–Lambert law. The dataset consisted of 2D STXM component maps. Images were $150 \times 150$ pixels at a pixel size of 50 nm.

The aim of the second analysis was to investigate the association of Fe and Cd in a system influenced by an Fe-reducing bacterium. Therefore, the bacterial strain Geobacter sp. Cd1 was grown in the presence of Cd-loaded ferrihydrite (an Fe(III) oxyhydroxide; Muehe et al., 2013b). The sample consisted of a dried bacterial cell suspension. SEM–EDX maps of $512 \times 448$ pixels were acquired using a LEOM VP 1540 equipped with an Oxford Inca Energy 200 Premium Si(Li) SATW detector at an acceleration voltage of 17 kV and a pixel size of 25 nm. The dataset, which is part of the data shown by Muehe et al. (2013b) consisted of EDX maps showing the distributions of Fe and Cd.

The third dataset was used to analyse the sorption behaviour of Fe$^{3+}$ to extracellular polymeric substances (EPS) in cell-mineral aggregates formed by BoFeN1 bacteria. In samples of a bacterial culture, EPS was labelled with a wheat germ
agglutinin – Alexa Fluor® 633 conjugate and ferric iron was labelled with an Fe$^{3+}$-specific probe (Zhang et al., 2011) as described by Schmid et al. (2014). Data were acquired using an upright Leica TCS SPE system (Leica Microsystems, Wetzlar, Germany). The voxel size was 23 × 23 × 168 nm$^3$. The dataset consisted of 3D CLSM maps representing the distribution of EPS and Fe$^{3+}$.

Usage of the plugin: evaluation of STXM dataset

Figure 1 shows a flow diagram describing the basic workflow. The diagram is illustrated with graphics produced during analysis of the STXM dataset and described in the following.

Image preprocessing

As a first step, the data has to be converted into the required form. For technical reasons, images have to be of the same dimensions and of 8-bit grey scale format. The images have to be aligned in a way that corresponding pixels in the two images represent exactly the same part of the sample. Images were exported from aXis2000 as 8-bit data. The grey values can be linearly converted into units of OD by multiplying with factors of 7.45 × 10$^{-3}$ (OC) and 6.12 × 10$^{-3}$ (Fe), respectively (see Supporting Information for details). Alignment was necessary as both images resulted from separate measurements. To restrict the analysis to the region of the cell-mineral aggregate while excluding the background, a mask was created using ilastik (Sommer et al., 2011) that was used to set pixels in the background area to zero in both images. Input images are shown in Figures 1(A, OC map) and (B, Fe map).

Optimizing scatterplot display

For generating a scatterplot, both input images have to be opened in ImageJ. After selecting the input images in the ScatterJ window, a scatterplot is automatically created and displayed using default settings (Fig. 1C). The scatterplot provides an overview on clustering, trends, and variation. Using the options provided under ‘Axis’ and ‘Colour scale’, it is possible to adjust the display of the scatterplot. After entering the previously mentioned calibration factors, the axes were scaled in units of OD. The positions at which tick marks should be drawn at the axes were selected by entering the respective values. In addition, the colour scale was adjusted as for the small number of data points it was not necessary to use the full range of colour grades. The optimized scatterplot is displayed in Figure 1(D). The selection of the next steps depends on the type of the data and the pattern and clustering of the scatterplot.

Backmapping

It is obvious that some of the data points are set apart from the main point cloud, forming a separate cluster in the upper part of the scatterplot. This cluster was highlighted by drawing a ROI in the scatterplot image (Fig. 1E). Backmapping produced a map (Fig. 1F) of the pixels in the input images that contribute to the highlighted cluster in Figure 1(E). Although this approach is not necessarily objective (Bonnet, 1995), it provides a direct way of obtaining qualitative but unambiguous information on the relationship between spatial localization and chemical composition. Thus, it is a powerful tool to identify regions in a sample of specific chemical composition that are not obvious in the original images.

Regression analysis

The shape of the point cloud suggests an overall linear trend. The input images contain the local concentrations of OC and Fe in quantitative units. Therefore, the presumed linear trend can be described using linear regression. As both variables were acquired using the same technique, a similar statistical error can be assumed. According to Rayner (1985), it is appropriate to use the MA regression method. An MA regression line was calculated for the whole dataset. Background pixels, represented by data points with coordinates (0,0), were not included in the calculations.

The pixels within the above-mentioned separate cluster appeared to follow a different trend; therefore a second regression line was calculated for the cluster. For MA regression, ScatterJ additionally permits to calculate and plot the minor axis (which is the axis perpendicular to the MA) and the 95% confidence ellipse of an assumed bivariate normal distribution. We used these additional options for analysis of the small cluster. The resulting structure helps to obtain an impression on the shape and orientation of the cluster. Figure 1(G) shows a scatterplot with the graphical results of the regression analyses.

Deviation maps

Despite the general linear trend, there is a considerable spread of the main point cloud. It is unclear whether this spread results from actual differences in composition or simply from statistical variation. We generated a deviation map showing the distance of data points in the scatterplot from the MA regression line. We chose the perpendicular distance, which corresponds to the (square root of the) parameter minimized by MA regression (Rayner, 1985). The map produced by ScatterJ shows, for each pixel, the deviation of the corresponding data point in the scatterplot from the MA regression line (Fig. 1H). This is represented using a colour scale. Background pixels, i.e. pixels with intensity zero in both images (see above), were excluded and are represented as a white background area in the deviation map, which greatly facilitates interpretation. It is important to note that the colour scale of the deviation map represents a different kind of information than the colour scale
Fig. 1. Basic workflow for data evaluation using ScatterJ. (A, B) input images (OC and Fe map), scale bar 2 μm. (C) Scatterplot using default settings. (D) Optimized scatterplot. (E) Highlighted cluster for backmapping. (F) Map of pixels contributing to the cluster in E. (G) MA regression lines for both the full dataset and the separated cluster. (H) Deviation map using MA regression line and orthogonal distance.

of the scatterplot. In the present case, it can be seen that regions of similar colour exist, representing similar deviations of the pixels from the regression line. Purely statistical variation should have led to randomly distributed colours in the deviation map. This means that the spread of the point cloud is indeed due to compositional variations within the sample.
Evaluation of CLSM stacks

CLSM images were corrected for noise using the AutoQuant\textsuperscript{TM} blind deconvolution algorithm (10 iterations) of the Leica software suite LAS AF. They were imported into ImageJ as 8-bit grey scale stacks. No further preprocessing was necessary.

ScatterJ was used to create a scatterplot from the two input stacks. As it did not show any obvious trends or clustering, a deviation map was created that shows the angular distance of the data points relative to the x-axis. The deviation map displays, for each pixel, the angle of the location vector of the corresponding data point with the x-axis. As a consequence, data points with the same Fe/EPS signal ratio are shown in the same colour. High angles (in red) stand for high Fe/EPS ratios: low angles (in blue) represent low Fe/EPS ratios. To show the three-dimensionality of the map, an orthogonal views representation (created using the 3D Viewer plugin for ImageJ; Schmid et al., 2010) is shown in Figure 2(E).

Evaluation of EDX maps

EDX maps of Fe and Cd were originally in 16-bit format. Due to low count rates, the EDX maps initially had maximum grey values considerably lower than 255, which is the maximum value for 8-bit images. Upon conversion to 8-bit, the histograms were stretched individually to cover the whole [0,255] range. As pixel sizes were smaller than the interaction volumes, there is a nonnegligible probability that an X-ray photon did not originate at the position to which it was assigned. We accounted for this by applying a Gaussian filter with $\sigma = 1$ pixel (25 nm). This had the additional effect of smoothing the histograms, as described by Muehe et al. (2013a). After filtering, the maps no longer represent the X-ray counts measured at the positions of the pixels. Instead, we consider them to show (uncalibrated) probability distributions for the respective elements. No alignment was necessary as the maps of the individual elements are acquired simultaneously.

Using ScatterJ, a scatterplot was generated. Backmapping was applied to analyse the two clusters visible in the scatterplot. The resulting maps were combined into a map showing the areas corresponding to the two clusters.

Results and discussion

Evaluation of STXM maps

The STXM maps (Figs. 1A,B) show a single BoFeN1 cell associated with some extracellular material. Despite minor internal variations, both maps show a structure of the same shape, indicating a close association of Fe and OC in all parts of the aggregate.

Grey values linearly convert into OD, which was accounted for by entering the respective factors into the plugin. The axes of the scatterplot are thus scaled in units of OD. The scatterplot (Fig. 1D) shows an overall linear trend with a small cluster of data points above the main cluster. Using the backmapping function, it could be shown that this cluster consists of ~450 data points and corresponds to a spatially coherent region in the maps (Figs. 1E,F).

A regression line (MA) was calculated for the whole set of data points (Fig. 1G). The overall correlation of Fe to OC follows a trend line with a slope of 0.88 in OD units, indicating a sorption process of Fe on the OC. Additionally, MA regression was performed for the small cluster highlighted in Figure 1(E), including minor axis and 95% confidence ellipse (Fig. 1G). For the small cluster, the slope of the MA turned out to be 2.43 in OD units. This means that the association of Fe and OC must be of a fundamentally different kind for that area, which is probably caused by a different sorption or mineralization (precipitation) process.

The heterogeneity observed in the deviation map (Fig. 1H) indicates that, in addition to the upper cluster, other regions of distinct chemical compositions exist. One example is the region represented by dark blue colour, which corresponds to parts of the bacterial cell. Within that region, Fe/OC ratios are considerably lower than the average described by the regression line. This might be caused e.g. by a different type of OC within the cell.

Evaluation of CLSM data

Single slices of the stacks representing EPS and Fe\textsuperscript{3+} distributions are shown in Figures 2(A) and (B). In both images, it is possible to distinguish cell-shaped as well as noncellular structures. Neither species is homogenously distributed among the cells or within the extracellular space. In addition, concentrations of both species vary strongly within the analysed volume, leading to a large spread of the fluorescence intensity signal. As a result, any further direct visual interpretation is difficult. Because the maps are 3D, it is not possible to display all the information at once.

To identify and localize sorption or complexation processes of Fe\textsuperscript{3+} within the bacterial extracellular polymer matrix, it is first necessary to test whether Fe\textsuperscript{3+} and EPS are colocalized, and if indicated, to identify regions of colocalization. The scatterplot (Fig. 2C) displays the relationship of both variables in 2D. It shows a point cloud stretching over almost the whole plot window. Potential trends or gradients, if they exist, are hidden within the data cloud.

To nevertheless test for the existence of compositional trends, i.e. spatially connected regions that show a similar chemical composition, we created a deviation map using the angular distance with respect to the x-axis (Figs. 2D,E). Now, it becomes obvious that indeed volumes exist that have rather homogeneous chemical compositions. The map highlights several clearly distinct regions, which represent volumes with homogenous composition, separated by small transitional areas. Some cellular shapes can be distinguished. The Fe\textsuperscript{3+}/EPS
signal ratio varies strongly between those cellular shapes. This clearly indicates the presence of cells at different stages of Fe$^{3+}$ sorption/complexation in their closely associated polymer matrix. This can be explained by a succession of increasing Fe$^{3+}$ sorption/complexation to the EPS matrix, preceding the formation of Fe(III) minerals, by different grades of association of bacterial cells with EPS (Schmid et al., 2014), or a different organochemical composition of the EPS in particular with respect to functional groups that can bind or complex Fe$^{3+}$.

In principle, a map showing the ratio of the signals would contain the same information as the deviation map. However, the ratio is poorly suited for displaying chemical trends in a scatterplot as it is very sensitive to changes in the denominator if the denominator is small. Thus, the signal ratio is much less sensitive to detail near the $y$-axis than near the $x$-axis. Using the angular distance in the scatterplot, which is basically the arctangent of the signal ratio, this problem is efficiently overcome. Our plugin thus is an excellent tool for the identification and visualization of chemical gradients and trends within a sample that are not obvious in large and complex 2D or 3D datasets.

**Evaluation of EDX maps**

Figure 3(D) shows a secondary electron image of the analysed sample area. Two particles are visible. The corresponding EDX maps represent the distribution of Fe (Fig. 3A) and Cd (Fig. 3B). Both particles contain significant quantities of Fe and Cd, but it is obvious that they have different average concentrations of the individual elements. However, the signals are not homogeneously distributed over the particle areas. Within the particles, signal intensities vary on different spatial scales. Thus, in the images it is not entirely clear whether both particles actually represent distinct phases.

The scatterplot (Fig. 3C) shows a point cloud that is separated into two clusters. The upper one (cluster 1) is defined by higher Cd and lower Fe concentrations as compared to the lower one (cluster 2). Cluster 1 is oriented vertically, indicating that the concentrations of Cd are independent from the concentrations in Fe. The slightly inclined shape of cluster 2, by contrast, suggests a certain interdependence between the concentrations of Cd and Fe. The clusters are clearly separated from each other, representing each a chemically distinct structure. Applying the backmapping function after the selection of the individual clusters produced maps that show in real space the areas contributing to the cluster. Figure 3(E) is an overlay of the maps obtained by applying the backmapping function for each cluster individually (cluster 1 in grey, cluster 2 in white). This image shows that both clusters originate from spatially coherent areas (Fig. 3E) that coincide with the areas occupied by the two particles in the secondary electron image.

This procedure shows that the two particles are distinct with respect to their chemical compositions and thus constitute different phases. Despite the strong internal inhomogeneities, no significant overlap of the respective clusters in the scatterplot.
Fig. 3. EDX maps of soil sample. (A) Fe map (generated from Fe-Kα signal). (B) Cd map (generated from Cd-Lα signal). (C) Scatterplot of Cd versus Fe (axes scaled in arbitrary units). (D) Secondary electron image of analysed area. (E) Combined results of backmapping: grey area corresponds to the upper cluster (cluster 1), white area corresponds to the lower cluster (cluster 2). Scale bar 2 μm.

is observed. Moreover, for the particle on the right, which corresponds to cluster 1, the concentration of Cd is independent from the concentration of Fe, whereas for the particle on the left (corresponding to cluster 2), some dependence might exist.

The maps presented in Figure 3 represent a dataset that is not well suited for quantitative analysis. However, it was possible to extract qualitative information that is much less ambiguous than visual interpretation. It is possible to use this method on datasets covering larger numbers of particles. In fact, it has been very successfully applied to detect changes in heavy metal/mineral associations in soil samples comprising a much greater number of particles (Muehe et al., 2013a).

Conclusions

The plugin presented in this paper is a powerful tool for extracting information from analytical microscopy datasets that might not be obvious or is well hidden in the data. We have shown this exemplarily using three fundamentally different datasets (2D STXM maps, 3D CLSM maps, 2D EDX maps) wherein the use of ScatterJ yielded novel information on processes that occurred in the biogeochemical samples. Although all of the presented examples originated from a specific field of research, the plugin can be used on various types of 2D or 3D analytical microscopy data that, e.g. represent elements, chemical species or other specific maps from any field of science.

Acknowledgements

The authors thank M. Muehe for providing soil samples, I. Adaktylou for providing data, O. Cirpka for discussion, the staff of the spectromicroscopy beamline 10ID-1 at Canadian Light Source for their support, Adam P. Hitchcock for discussion, and two anonymous reviewers for their very constructive suggestions. The tutorial on writing ImageJ plugins by W. Bailer (http://www.gm.fh-koeln.de/~konen/WPF-BV/tutorial-ImageJ_V1.71.pdf) was a great help. This work was funded by DFG Emmy-Noether program to M.O. (OB 362/1–1). The Canadian Light Source is supported by NSERC, CIHR, NRC, the Province of Saskatchewan, WEDC, and the University of Saskatchewan.

References


