Reconstruction of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) spatial distribution images in Microsoft Excel 2007

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A B S T R A C T

The spatial distribution of metals in biological tissue is an important field in biomedical research. Based on the fundamental significance of metals in health, many metal imaging analysis methods were developed during the last decades. Metal and non-metal imaging by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is an effective mean to obtain data in high spatial resolution and sensitivity. Unfortunately, the visualization of metal measurements normally needs special software solutions. This work reports the development of a new software tool allowing the reconstruction of metal distribution maps using Microsoft Excel 2007 with the aid of Visual Basic for Applications (VBA) user-defined functions. This routine uses continuous lists of measured data points as raw input. A key step is the determination of the correct length of measurement lines to constitute images without shearing. Adaption to special requirements is easy. It permits the fast generation of high quality scaled images from Microsoft Excel. Moreover, the visualization tool is simply transferable to other systems and can export the images in common image formats such as TIFF or JPG which then can be read out by established image treatment and region of interest handling software such as Image J.

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1. Introduction

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is today a well established technique for direct analysis of solid samples [1,2]. It allows the uncomplicated elemental analysis of many different kinds of sample material with inexpensive preparation methods. It can be used for the precise determination of major, minor and trace element distributions or isotope ratios and is therefore already applied in many research fields [3,4]. The technological progress in this field has allowed to measure metals in the ppm-range and below in a large variety of biological samples while maintaining the native biochemical environment and physiological state with high selectivity and spatial resolution [5].

In contrast to MALDI-MSI, in LA-ICP-MS ablation of a fixed raster of points ("shot to shot") never became standard. Rather more,

the sample is scanned line by line ("fire on the fly") whereby the material is entirely ablated throughout the depth under continuous movement of the sample stage into x-direction. Due to the fact that synchronization of LA apparatus, respectively laser shots and ICP-MS data acquisition has to be individually adapted to the dead volume of the ablation chamber, tubing and carrier gas flow, this technique was never successfully implemented in routine. Data acquisition with continuous stage movement brings the advantage that there is no delay time between the acquisition of single data points and therefore, desynchronization errors are excluded. As it would be the case in an oversampling raster, all the material is exploited for analysis, fractionation effects are minimized and measured values are true averages throughout the sample depth. Although the sample depth varies from point-to point in dried sections, it corresponds to a constant thickness of wet material before cryo-sectioning. Therefore, the reconstruction of images provides some challenges. First, image pixels are almost never ideally quadratic, but the x width corresponds to the product of x speed of the sample stage and the acquisition time for one mass spectrum which is the sum of dwell times for all selected elements. The y-pixel length is the laser spot diameter plus the chosen distance between lines. Exact proportion of x/y dimension can be assured by

spatial coregistration to a microphotograph obtained before ablation. Next, ideally the data of each line are written as a separate data file. Then single lines have just to be stacked together to constitute the 2D-image. Several software routines for this purpose have been published. However, this is not implemented in every set-up and there is a non-negligible risk of software hang-ups during data acquisition, for example, of an overnight analysis run in certain set-ups. Therefore, in this and many other works, data of subsequent lines were written as a continuous stream in a single file. To reconstruct the 2D-image, this stream has to be split again into single lines. Due to the lack of synchronization, the length of individual lines varies by one pixel and mostly equals a non-integer number in average. Manual arrangement of data into lines would be very time-consuming. Therefore C++ or Matlab [6–8] based software tools have been designed, where only the line length has to be entered once manually [9]. Others used Origin [10], Python-based ISIDAS with Maya Vi2 [11] or the SMAK-package [12] as software for LA-ICP-MS image treatment. As these "idioms" have few "native speakers" in the biomedical community and adaption to the special needs of individual datasets is desirable, the analysis of the measurements is mostly performed in Excel. Therefore, there was an urgent need to create an entirely Excel-based solution.

We here present a new Microsoft Excel 2007-based software tool with user-defined functions in Microsoft Excel VBA, hereafter referred to as ELAI. This software allows the generation of images from mass spectrometry data, offering the potential for free customizable semi-manual image generation without any further additional commercial software. The parametric images generated can be transformed and exported into common image formats such as TIFF or JPG.

2. Material and methods

2.1. Sample preparation

All mice used in this study as organ donors were housed at the University Hospital Aachen and received humane care. The animal protocols were in full compliance with the guidelines for animal care and approved by the responsible German Animal Care Committee (LANUV, Recklinghausen, Germany). The respective permission has the identification number 84.02.05.2011.265. Mice organs (i.e. brains and livers) were cryo-cut into 30 μm slices with a cryomicrotome (CM3050S, Leica Biosystems, Wetzlar, Germany) on −18 °C cryo-chamber temperature and −16 °C object area temperature, thaw-mounted onto adhesive StarFrost® microscope slide (Knittel Glass, Braunschweig, Germany), dried and stored at room temperature.

2.2. Measurement set-up and LA-ICP-MS measurements

Before sample analysis, the tissues were photographed at 4× magnification with a microscope (IX81, Olympus, Hamburg, Germany) to subsequently allow easy assignment of image dimensions.

LA-ICP-MS measurements were performed using a quadrupole ICP-MS (XSeries 2, Thermo Fisher Scientific, Bremen, Germany) without operating the collision cell. The Nd:YAG laser ablation unit (NWR260, New Wave, Fremont, CA, USA) was directly connected to the ICP torch. The measurement parameters (Table 1) including RF power and gas flow were optimized for each sample run with a daily performance test. To keep the measurement time of the mass spectrometer as low as possible and for receiving a high spatial resolution of the image, only isotopes of interest were selected. The dwell time was adjusted to the expected concentration of each selected isotope.

Table 1
Common LA-ICP-MS measurement parameters.

Parameter	Value range
RF power	1400–1600 W
Carrier gas flow	0.85–1.05 L/min
Number of isotopes	21–29
Dwell time per isotope	0.02–0.3 s
Laser power	50%
Scan speed	60–90 μm/s
Spot size	30 μm
y-distance between the centre of lines	30 μm

In the ablation apparatus, autofocus adjustment of the z-stage position at the starting and ending point of every line was selected. The ablation was performed line by line with a continuous motion in the x-direction and a discontinuous motion in the y-direction while sample material was continuously transported to the mass spectrometer. The primary output data were a continuous list of ion intensities with no trigger signal indicating line shifts because the mass spectrometer operated independent from the laser unit. In some cases the mass spectrometer refused to record more than 32,768 data points (16 bit) in one raw data file. To prevent data loss, the raw data were split into two or more files if the expected number of data points exceeded this number. When creating the next raw data file, the recording stopped for about 1 min while the laser continued ablating the sample material. These data points were lost and had to be interpolated in the data analysis.

Before each measurement the LA-ICP-MS had to be equilibrated to obtain constant plasma conditions and warm up the laser. Therefore, the ablation chamber was washed by constant carrier gas flow for about 1 h to remove air.

In some datasets y-drifts occurred when the equilibration time of the system or the washing time of the ablation chamber was too short. Comparatively weak y-drifts were also found in long lasting measurements, i.e. over 12 h. Rarely x-drifts occurred due to imperfect laser focus adjustment.

2.3. Calibration and standard preparation

To obtain element concentrations, a calibration with standards is needed, which matches the sample matrix and covers a wide value range for the elements of interest. Ideally, the calibration material for biological samples is produced of the same tissue type and the same species to ensure consistent ablation behaviour [13], especially in regard to the water content that – depending on its extent – may have a huge influence on the element concentrations. Standards were produced from homogenized tissue spiked with varying concentrations of a standard salt solution. In brief, for the production of one calibration standard, about 1 g of tissue was needed. Normally, five calibration standards were spiked with different amounts of the standard salt solution and one or two blank standards were produced from 7 g pure tissue. The organs were homogenized with a speed tissue homogenizer (Ultra-Turrax TP 18/10, IKA, Staufen, Germany) at 20,000 rpm for about 1 min. About 1 g of the homogenized tissue was weighed exactly into a 2 mL micro reaction tube for each calibration standard.

The salt solution used for spiking the standards was produced from different salts and acids containing the elements of interest using the highest available purity (A1. Suppl. Table 1). Overnight, a white precipitate had formed which was centrifuged and separated. The supernatant was added in increments of 200 μL, 100 μL, 50 μL, 25 μL and 10 μL to the micro reaction tubes containing the homogenized tissue. The absolute concentration of each metal was determined taking into account the individual recovery rates that were highly variable and in the range of 7% and 93%. Subsequently, each tube was rigorously mixed and transferred into a plastic mould