

## Motivation

Hyphenating laser ablation (LA) to inductively coupled plasma mass spectrometry (ICP-MS) has evolved to a major technique in direct elemental analysis with high spatial resolution. However, strong matrix effects, elemental fractionation and the lack of available standard reference materials strongly limit an accurate calibration for quantitative analysis in many applications. The aim of this study is to develop a calibration method for the analysis of discrete  $\mu\text{m}$ -sized, such as single particles or cells.

## Concept

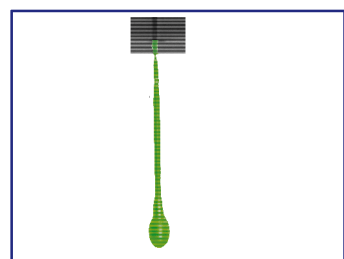


Fig.1: Precise transfer of pL volumes of standard solution onto solid sample (microscope image included in graphic illustration).

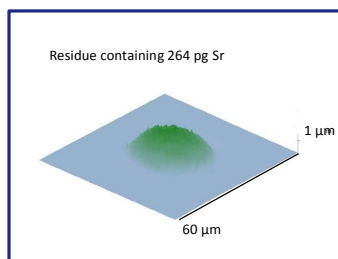


Fig.2: AFM image of a residue (after drying) with known total mass and diameter in the  $\mu\text{m}$ -range.

The proposed calibration strategy (Fig. 1-4) is based on the total ablation of dried residues from pL droplets with known volume of standard solutions.<sup>1</sup> Therefore, a novel drop-on-demand aerosol generator based on thermal inkjet technology was designed, which can also be applied to the reproducible transfer of minute amounts of sample mass onto various sample targets.<sup>2</sup> After drying, the obtained residues can be completely ablated for calibration in ICP-MS.

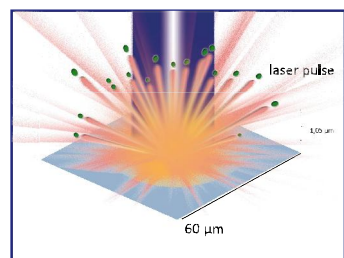


Fig.3: Complete ablation of individual dried residues of standard solution (laser beam diameter > object size).

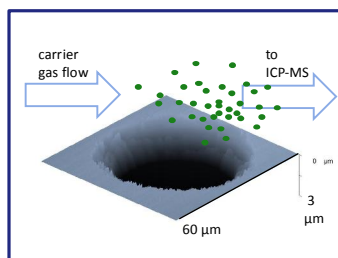


Fig.4: AFM image of an ablation crater. Transportation of the aerosol to the ICP-MS results in transient signal.

## Literature

- [1] U. E. A. Fittschen, N. H. Bings, S. Hauschild, S. Förster, A. F. Kiera, E. Karavani, A. Frömsdorf and J. Thiele, *Anal. Chem.*, **2008**, *80*, 1967-77.
- [2] J. O. Orlandini v. Niessen, J. N. Schaper, J. H. Petersen and N. H. Bings, *J. Anal. At. Spectrom.*, **2011**, *26*, 1781-1789.
- [3] U. E. A. Fittschen and G. J. Havrilla, *Anal. Chem.*, **2010**, *82*, 297-306.
- [4] J. H. Petersen, J. O. Orlandini v. Niessen, J. N. Schaper and N. H. Bings, 37th FACSS Conference, Raleigh, NC (USA), **2010**, poster.
- [5] P. H. Pacheco, R. A. Gil, S. E. Cerutti, P. Smichowski and L. D. Martinez, *Talanta*, **2011**, *85*, 2290-2300.

## Experimental Setup

A novel interface has been designed (Fig. 5) to minimize the previously observed influence on the precision (transferred mass per dosing event) caused by the humidity of the ambient air and the idle time between two dosing events.<sup>3,4</sup>

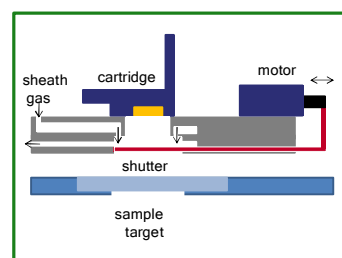


Fig.5: Schematic illustration of the novel interface, which allows to control the humidity of the sheath gas and to automatically discard selected droplets.

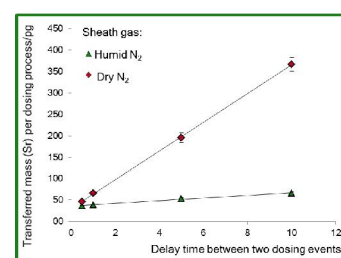


Fig.6: Influence of the delay time between two dosing events on the transferred mass per dosing event (mean value of 200 events, determined by ICP-MS). Comparison between dry and humid (80 % relative humidity) nitrogen.

The addition of humid sheath gas (Fig. 6) attenuates the dependency of the delay time between two dosing events on the transferred mass per dosing event. The motor driven shutter allows to automatically discarding selected droplets, especially such first droplets with irreproducible and biased sizes, which are observed in the beginning of each dosing sequence. Therefore the novel interface increases the reproducibility of the transferred masses.

## Fundamental Characterization

TXRF (Fig. 7) and LA-ICP-MS (Fig. 8) studies were carried out on multiple residue layers of a 1 g/L Sr standard solution (2,4,8 and events). For both analytical techniques linear calibration curves with good correlation coefficients and low relative standard deviations were obtained.

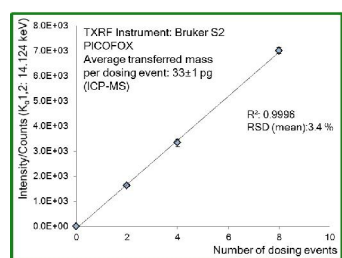


Fig.7: TXRF calibration curve for Sr using residues of dried droplets analysis (integration time 1000 s).

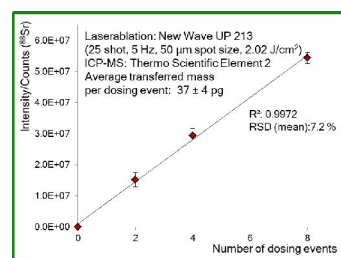


Fig.8: Calibration curve (Sr) for LA-ICP-MS analysis (integrated transient signals) based on total ablation of dried residues from DOD (integration of transient signals).

Tab. 1 & 2 summarize the results of TXRF and ICP-MS studies using a 1 g/L multi-element solution.

Tab. 1: Average transferred mass per dosing event determined by ICP-MS (200 dosing events).

Element	m/z	transferred mass per dosing event	SD
Cr	53	28	1.5
Mn	55	27	1.3
Co	59	25	1.1
Cu	63	27	3.0
Ga	69	23	1.0
Sr	88	25	1.4
Ag	107	23	1.1
In	115	23	1.4
Ba	137	25	2.4
Tl	205	23	1.7
Pb	208	25	3.0
average		25	1.9

Tab. 2: LODs based on TXRF calibration curves using a multi-element standard solution. The mean transferred masses were previously determined by ICP-MS.

Element	Line	Energy /eV	R <sup>2</sup>	RSD (mean)	LOD /pg
Cr	K $\alpha_{1,2}$	5.415	0.9998	6.3%	7.6
Mn	K $\alpha_{1,2}$	3.314	0.9996	5.6%	4.5
Fe	K $\alpha_{1,2}$	6.405	0.9997	4.8%	6.5
Co	K $\alpha_{1,2}$	6.931	0.9996	5.0%	2.0
Ni	K $\alpha_{1,2}$	7.480	0.9998	4.8%	3.0
Cu	K $\alpha_{1,2}$	8.046	0.9996	3.8%	3.7
Zn	K $\alpha_{1,2}$	8.637	0.9999	3.9%	7.9
Ga	K $\alpha_{1,2}$	9.251	0.9999	3.8%	0.9
Sr	K $\alpha_{1,2}$	14.165	0.9997	4.0%	1.7
Ba	L $\alpha_{1,2}$	4.466	0.9954	15.1%	36.5
Tl	L $\alpha_1$	10.269	0.9996	5.6%	2.4
Pb	L $\alpha_1$	10.551	0.9997	5.7%	2.5
Bi	L $\alpha_1$	10.839	0.9998	4.6%	0.6

## Matrix-Matching Calibration for Single Particle Analysis by LA-ICP-MS

A matrix matching calibration for particles containing U as major and Pb as a minor compound was developed using the suggested DOD technique. Four different solutions were used, all containing 925 mg/L U and 5, 10, 20 and 40 mg/L Pb, respectively. Residues stemming from 2, 4, 8, and 16 dosing events of the ablation were ablated. For each solution the transferred mean mass (200 dosing events) was determined by ICP-MS. All calibration curves for <sup>238</sup>U match each other (Fig. 9) and show good linearity.

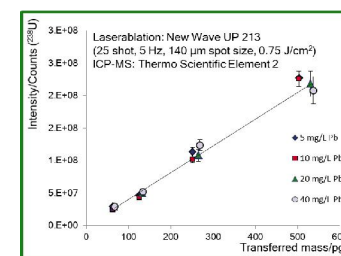


Fig.9: Calibration curve for LA-ICP-MS analysis of the major compound (U) of dried residues from DOD (Detector mode: analog).

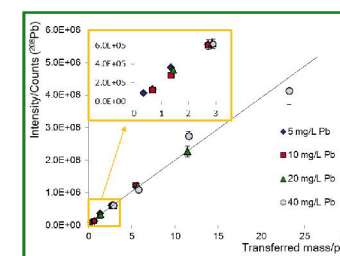


Fig.10: Calibration curve for LA-ICP-MS analysis of the minor compound (Pb) of dried residues from DOD (Detector mode: pulse counting).

LA-ICP-MS intensities for <sup>208</sup>Pb as the minor compound of the used model solutions of different concentrations very well match one calibration curve (Fig. 10). No significant effect of the U matrix (U: Pb ratio) was observed.

## Outlook I: Single Particle Analysis

Particles of two different bulk samples were immobilized on double-faced adhesive tape (Fig. 11) and analyzed by LA-ICP-MS. The obtained intensities are given in Tab. 3. For technical reasons no adequate quantification was so far possible, but the measured intensities of <sup>208</sup>Pb and <sup>238</sup>U are within the range of the previous calibration curves presented above. Thus, the proposed calibration technique should be applicable to LA-ICP-MS analysis of individual particles.

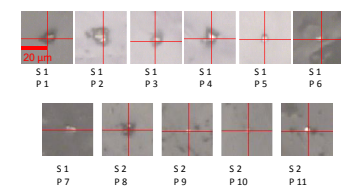


Fig.11: Microscopic images of the different analyzed Particles (P) from both samples (S)

Tab. 3: Measured diameter and Intensities of single particles containing U and Pb.

Sample	Particle	Diameter / $\mu\text{m}$	I (cps)	
			( <sup>238</sup> U)	( <sup>208</sup> Pb)
Sample 1	P 1	10 x 15	2.68E+05	2.51E+07
	P 2	10.0	3.29E+05	5.57E+07
	P 3	15.0	1.57E+05	2.03E+08
	P 4	10.0	3.81E+05	1.93E+07
	P 5	14.0	2.76E+05	6.98E+07
Sample 2	P 6	6.0	9.07E+04	1.95E+07
	P 7	6.0	1.03E+05	1.59E+07
	P 8	11 x 5	1.31E+06	3.91E+07
	P 9	7.0	1.016E+05	2.554E+07
	P 10	3.5	1.436E+05	4.803E+07
	P 11	3.5	1.963E+04	1.224E+07
	P 12	5.0	7.795E+04	2.775E+08

## Future Work

- Validation
  - Since no adequate reference material for the validation of the proposed single particle analysis technique is to date available, we suggest and plan to use individual, size-classified particles resulting from milling highly homogeneous glass standard reference materials (NIST 610 and 612), which are commonly used for LA-ICP-MS micro analysis. The particle mass can be derived from the particle size and the known density of the NIST reference material.
- Quantitative analysis of single particle and cells

## Outlook II: Single Cell Analysis

The biosorption of metals to inactivated cells of bacteria, algae and fungi is known to be very efficient and leads to a high enrichment in the applied biomass.<sup>5</sup> For a first approach, yeast cells of *Saccharomyces ludwigii* were investigated, as they can be easily cultivated with high biomass yield. Furthermore, the size of these cells is in average around 10  $\mu\text{m}$ , which meets two prerequisites: the objects need to be small enough to be fully exposed to the laser beam (spot diameter) but also large enough to be visible through the magnification optics of the LA system. Depending on the concentration of the redispersed, lyophilized cells, both single cells (Fig. 12) or thin films of cells (Fig. 13) can be studied.

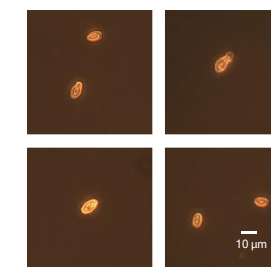


Fig.12: Microscopic images of segregated *S. ludwigii* cells after pipetting and drying (1  $\mu\text{L}$  of 1 mg/L dwf) on Makrolon® substrates.

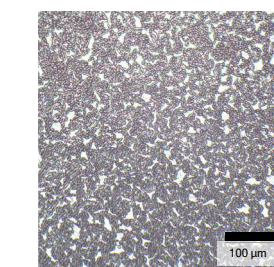


Fig.13: Microscopic image of a film of *S. ludwigii* cells after pipetting and drying (1  $\mu\text{L}$  of 1 g/L dwf) on Makrolon® substrates.

The biosorption of Hg, Pb and Zn (Fig. 14) was studied applying different concentrations of standard solutions. After washing the cells, direct TXRF for quantification was applied (no digestion, unfiltered and filtered < 0.45  $\mu\text{m}$ , internal standard: Ga). Total adsorbed masses per cell were calculated based on microscopically determined cell densities. Total concentration increases by a factor of more

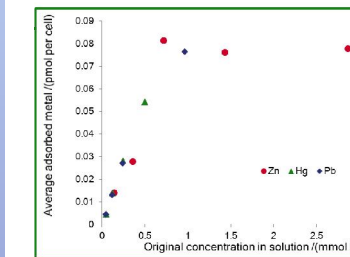


Fig.14: Biosorption of metals on *S. ludwigii* cells.

than 1000 compared to the original solution, while total adsorbed masses reach the low pg range. This makes the investigated cells an ideal model for further LA-ICP-MS-based quantitative cell analysis using the proposed DOD calibration technique.

## Conclusion

Dried residues of pL volumes of standard solutions generated by the novel thermal inkjet-based drop-on-demand dosing technique allow for a new matrix-matching calibration strategy in LA-ICP-MS, which shows the potential for application to quantitative single particle and cell analysis.

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