Supplemental information for

Quantification routines for full 3D elemental distributions of homogeneous and layered samples obtained with laboratory confocal micro XRF spectrometers

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A. Sample details

Table S1: Composition and measurement details of the examined samples.

	BLiX setup	Freiberg setup
One element samples (Bruker Nano calibration palette)	Ag, Al, Au, Bi, Cd, Cr, Cu, Fe, Ge, Mo, Ni, Pb, Pd, Pt, S, Si, Sn, Ta, Ti, Zn, Zr	Ag, Al, Au, Bi, Cd, Cr, Cu, Fe, Ge, Mn, Mo, Ni, Pb, Pd, Pt, Si, Sn, S, Ta, Ti, Zn, Zr
Thin foils [thickness / µm]	Ag [12.5] Ni [12.5] Pb [2] Zn [12.5]	---
Multi-element glass samples [density / g/cm³]	Breitländer GmbH: A4 [3.01] B3 [3.11] C3 [2.41] D3 [2.76] E3 [2.75] F3 [2.52]	NIST 610 [2.21] BAM S005c [2.46]
Standard reference materials [density / g/cm³ | measurement parameters]	NIST 697 Bauxite [1.93 | 40 s, 10 µm] NIST 1577 Bovine Liver [1.10 | 120 s, 10 µm] NIST 1646 Estuarine Sediment [2.00 | 40 s, 10 µm] BCR 129 Hay Powder [1.07 | 20 s, 5 µm] BCR 176R Fly Ash [2.00 | 20 s, 5 µm] BCR 667 Estuarine Sediment [1.99 | 20 s, 5 µm] CC 144 Sewage Sludge [1.24 | 20 s, 5 µm]	---
Multilayer [density / g/cm³ per layer | measurement parameters]	ZnO B [0.91,0.89,0.91,0.89,0.91 | 20 s, 10 µm] ZnO K [0.92,0.87,0.92,0.87,0.92 | 20 s, 10 µm] ZnO L [0.90,0.87,0.90 | 20 s, 5|10|20 µm]	---
Smoky quartz with goethite needle [densities / g/cm³ | measurement parameters]	---	[2.65, 4.28 | 10 µm 0.1 s/point]

B. Software details

i. *SpecFit* – Deconvolution Software

The crucial information for XRF is the net peak intensity of fluorescence lines. To evaluate these intensities the software *SpecFit* performs a deconvolution on the measured spectrum. The deconvolution is performed in 3 steps. First the spectrum can be either smoothed or stripped or both to erase the fluorescence peaks and derive an approximated background. In the second step the calculated background is subtracted from the measured spectrum and finally the fluorescence peaks are fitted with a Gaussian normal distribution to evaluate their net peak intensity. The fitting process can be performed linear with only the net peak intensity as fitting parameter or in a non-linear fashion.
In the nonlinear fit the energy scale is adjusted either for a single representative spectrum or for every single spectrum in a measurement. As the computing time can be long especially if many fluorescence lines are included, it is not advisable to fit many thousands of spectra of the same measurement in this mode. The non-linear fit is therefore typically performed in the beginning of the evaluation using the sum spectrum of the full measurement to optimize the energy scale.

In the linear-fit modus, *SpecFit* can deconvolve a large number of spectra rapidly. A fit of 20 fluorescence peaks can be performed with a deconvolution speed of 1250 spectra per minute, or approximately 20.8 per second. The deconvolutions were performed on a machine with 3.2 GHz Quadcore and 8 GB RAM. This computation is 13 times faster than commercially available software (Esprit (version 1.5.1.13) of Bruker Nano GmbH processes 1.6 spectra per second) and thus facilitates in principle on-the-fly deconvolution for future analysis. The net peak intensities for each fluorescence line are returned as NumPy arrays which can be further processed.
SpecFit is developed in Python. The deployed version uses Python 3.7.8 and NumPy 1.19.5 for mathematical array operations and SciPy 1.6.3 for optimization.

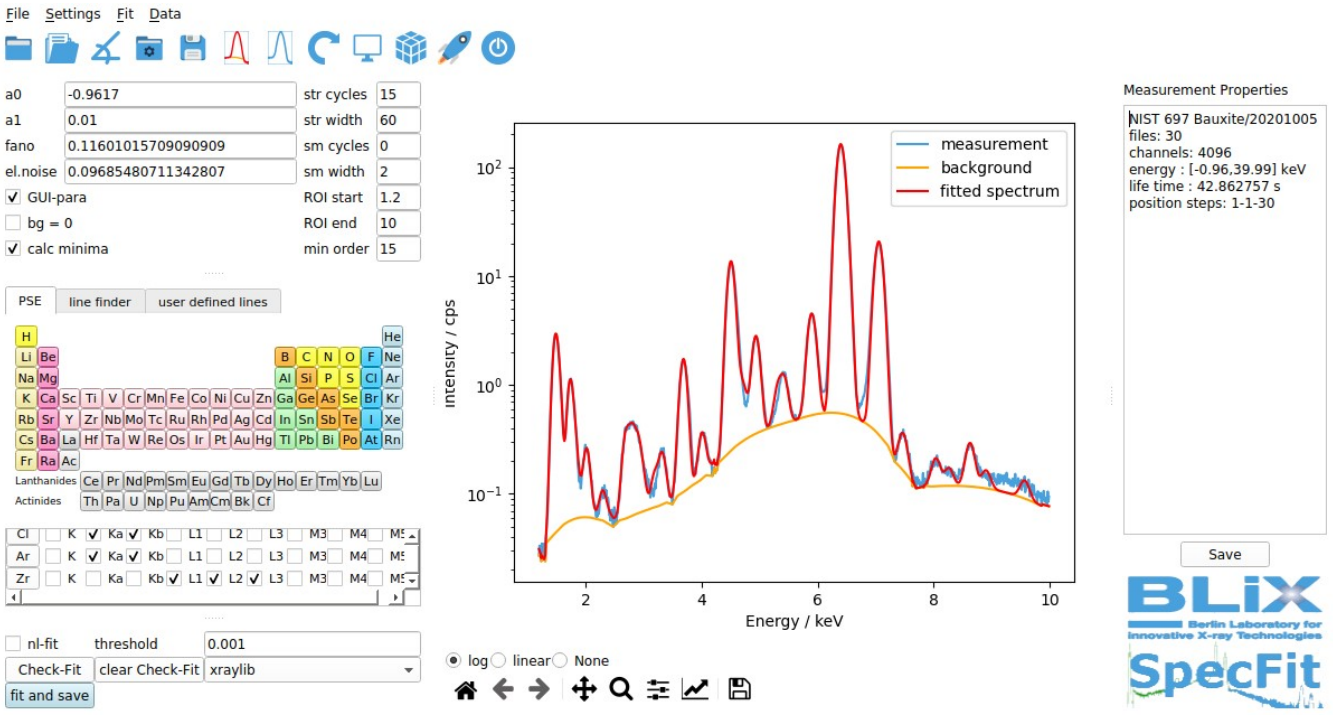

Figure S1: Graphical user interface (GUI) of the deconvolution software SpecFit. In the left panel energy calibration and smoothing parameter can be manipulated and element fluorescence lines can be selected. Fit results are displayed in the centered plot. On the right side measurement properties are listed.

ii. *AbsCor* – Calibration & Quantification Software

For the calibration of a confocal setup and quantification of measurements the equations (1) and (6) are implemented in *AbsCor* alongside visualization and manipulation routines.
In the calibration routine 15 parameters can be optimized, namely the surface position x_0, the thickness of the sample D, the amplitude A of the combined transmission, the width $B_{E|D}$ of the transmission, the center position $M_{E|D}$ of the transmission, the excitation and detection angles $\vartheta_{E|D}$, the maximum spot size $\sigma_{E|D}^{MAX}$ of the lenses, the exponential factor $\sigma_{E|D}^{exp}$ of the lenses and the minimum spot size $\sigma_{E|D}^{opt}$ of the lenses. Each one of them can also be fixed. With this flexible approach possible pre-characterizations of the first lens can be considered. To calibrate a setup multiple depth profiles, at least a number equivalent to the number of free parameters, are necessary. Different fluorescence lines of various standard reference materials can be processed, either from a single depth profile measurement or using the averaged depth profile from a 3D measurement for enhanced signal to noise ratio (SNR) and overall averaged elemental distributions. Performing the latter, an exact flat surface of the utilized SRM must be guaranteed to avoid higher errors in the fitting routine. The fit then minimizes the difference between measured and calculated depth profiles based on equation (1) of the main text. The underlying local or global minimization routine can be selected from a set of 18 different algorithms. The optimizers are implemented from the Python package *lmfit.* For local optimization the optimization algorithm *least_squares* is set as default and *basinhopping* for global optimization. The depth profiles can be normalized if needed.
When the optimization converges, the optimized values of the calibration parameters and the estimated covariance matrix are returned. The parameters are directly implemented in *AbsCor* and can be saved for future use. The optimization is terminated when the change in either the utilized cost function, the independent variables or the gradient is below a certain threshold.
The overall fitting procedure with a *least_squares* routine is dependent of the number of depth profiles and the starting parameters. Global approaches ultimately result in longer calculation times and would put a higher demand on the computer in terms of RAM. The calibration performed in this work took about 20 minutes on a 3.2 GHz Quadcore and 8 GB RAM computer.

With a calibrated setup an unknown sample can be quantified. For this purpose, the sample model must be defined. A tool for the definition of bulk and multilayer samples by defining their density, thicknesses and elemental concentrations is included in *AbsCor*. The quantification time is dependent on the accuracy of the starting parameters and the predefined sample model. To certify the defined sample and reduce the calculation time, forward calculations can be performed and the results are visualized to further adjust the sample model. While quantifying the concentration, every depth scan is handled individually with the assumption, that the neighboring depth profiles are equal. Inhomogeneities such as dents or element accumulations which directly affect the measured depth profile can be visualized but are not considered in the absorption calculation. Thus, the quantification is primarily reliable for lateral homogeneous bulk or stratified samples, or inhomogeneities with little effect on the overall absorption such as the measurements presented in the article.

A *least_squares* fit is then performed on every depth profile adjusting - if selected - the surface position x_0, the thickness of the sample or the positions of the layer boundaries and the concentrations. The latter results in a change of the absorption coefficient μ_{lin} which is constantly updated while the fit is running. When the optimization succeeds, meaning the change in either the cost function, the independent variables or the gradient is below a certain threshold, a concentration distribution of the sample is displayed and the concentrations, surface positions x_0 and thicknesses are returned as NumPy arrays. They can be further analyzed using either *AbsCor* or other software.

To review the calibration and quantification process the fitted and calculated depth profiles and parameters are constantly updated and displayed, allowing a continuous control of the parameters and results. Additionally, the loaded measurement can be manipulated by rotating, cropping and expanding to comply with the measurement and sample geometry.

AbsCor is developed in Python. The deployed version uses Python 3.7.8 and utilizes NumPy 1.19.5 for mathematical array operations and lmfit 1.0.2 for optimization.

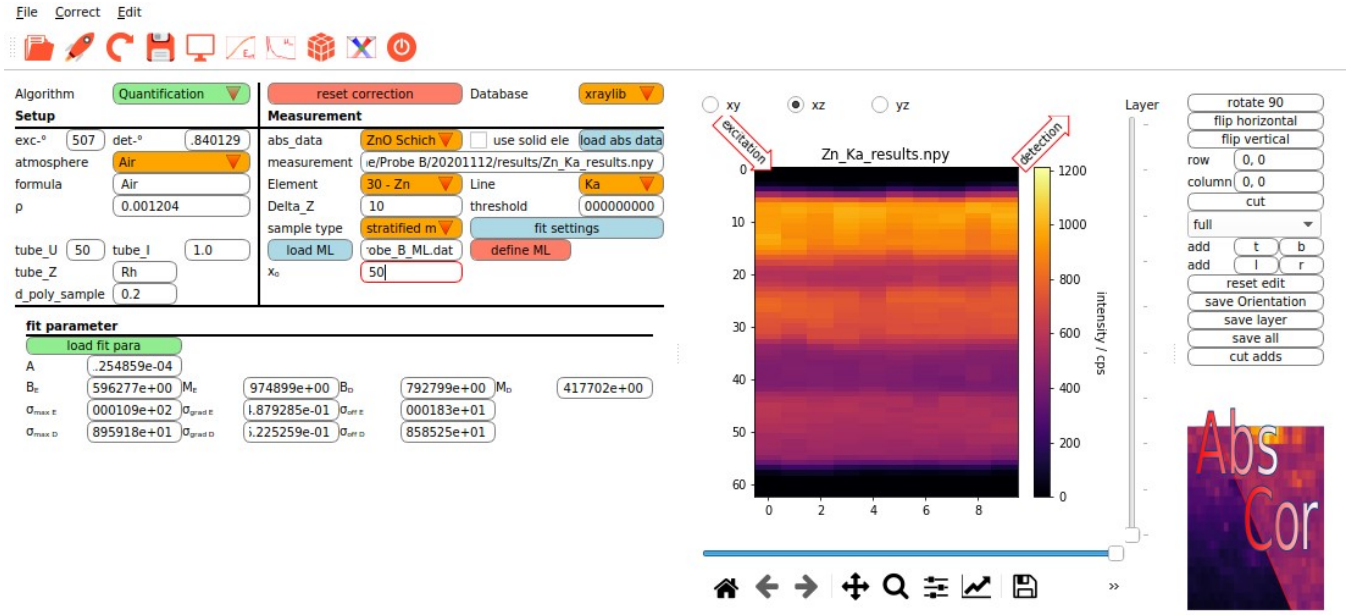

Figure S2: GUI of the absorption correction and quantification software AbsCor. In the left panel measurement, setup and sample parameter can be manipulated. Fluorescence intensity distributions or corrected/quantified distributions are displayed in the center. On the right side the loaded data can be manipulated to comply with measurement conditions.

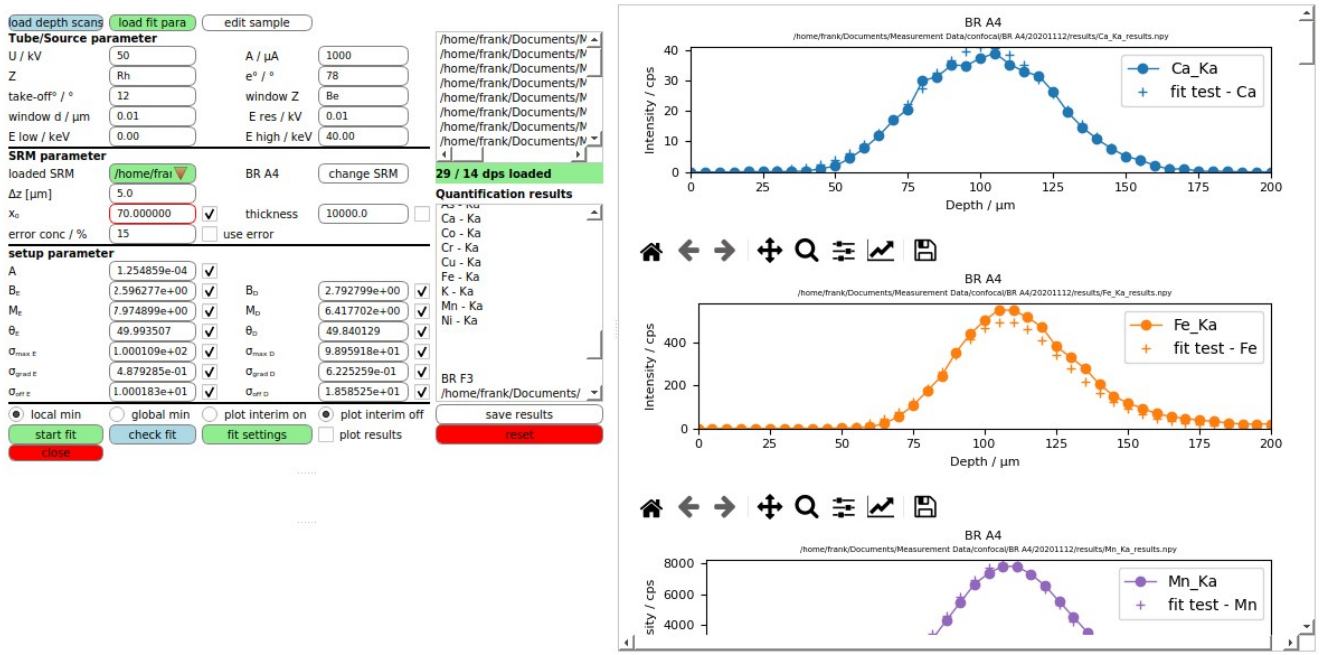

Figure S3: GUI of the confocal calibration software implemented in AbsCor. In the left panel measurement, setup and reference material parameter can be manipulated. Measured and calculated depth profiles are displayed on the right side.

C. calibration details

Table S2 Parameters for the calibration of the two CMXRF setups.

Parameters	BLiX setup	Freiberg setup
$A_{0,E}$ / cm²/g	0.066	0.073
$A_{0,D}$ / cm²/g	0.003	0.042
B_E / keV	3.893	5.547
M_E / keV	7.837	7.845
T0_E	0	0
B_D / keV	2.507	5.434
M_D / keV	6.564	10.174
T0_D	0	0
σ_E^{max} / µm	26.750	35.966
σ_E^{exp} / 1/keV	0.083	0.167
σ_E^{obj} / µm	4.479	19.385
σ_E^{max} / µm	33.309	59,645
σ_D^{exp} / 1/keV	0.194	0.385
σ_D^{obj} / µm	6.846	1.550

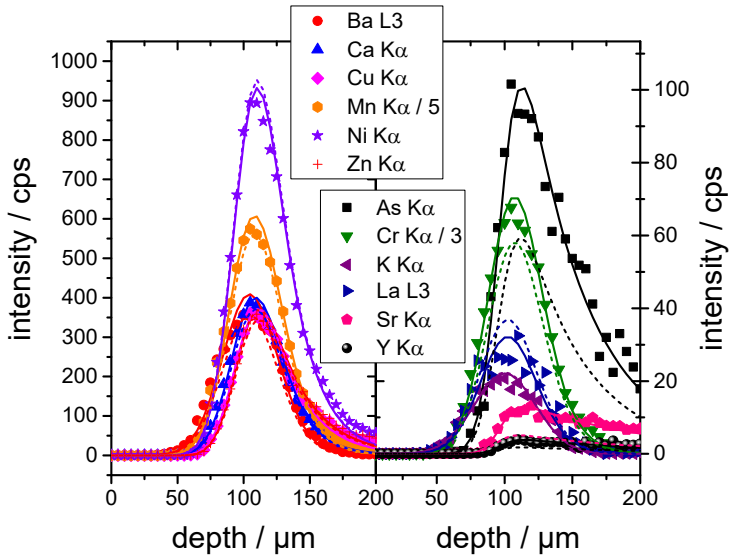

Figure S4: Calibration and quantification results of sample E3 analogue to figure 1 in the main text.

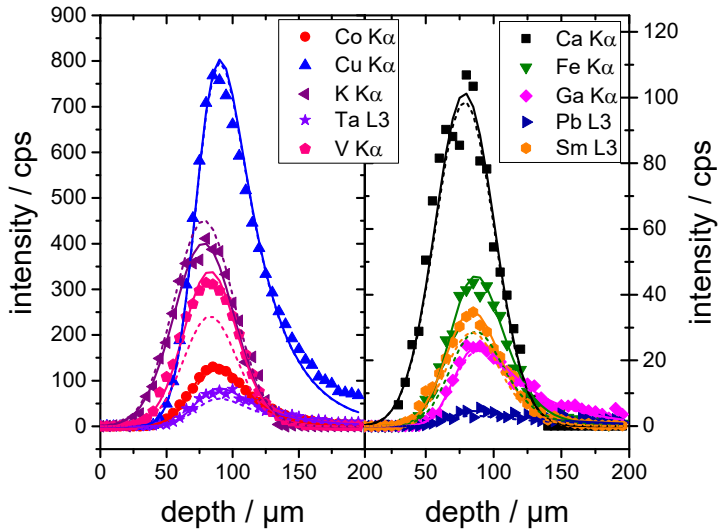

Figure S5: Calibration and quantification results of sample F3 analogue to figure 1 in the main text.

D. Step size evaluation

	5 µm		10 µm		20 µm		
ZnO L	conc/ %	D / µm	conc/ %	D / µm	conc/ %	D / µm	cert.
Layer 0	2.82 ± 0.02	64.9 ± 0.5	2.83 ± 0.03	64.7 ± 0.8	2.83 ± 0.04	64.6 ± 1.2	3.08
Layer 1	0.12 ± 0.03	54.4 ± 0.7	0.12 ± 0.04	54.8 ± 1.0	0.12 ± 0.06	54.5 ± 1.6	0.00
Layer 2	3.35 ± 0.04	43.3 ± 0.7	3.40 ± 0.04	42.5 ± 1.0	3.31 ± 0.09	43.2 ± 1.5	3.08

Table S3: Quantification results for a depth profiling measurement on sample ZnO L with varying depth step widths.

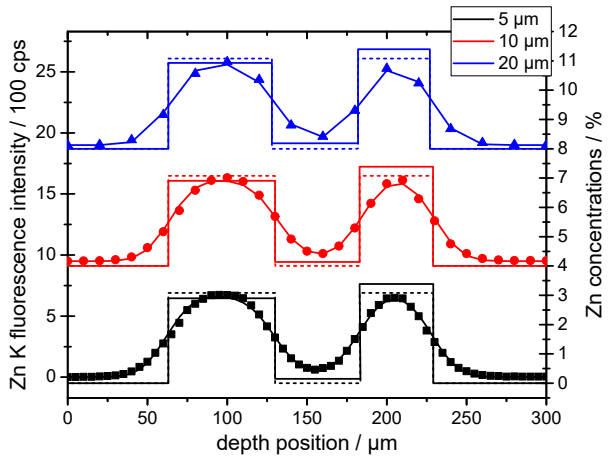

Figure S6: Depth profiling measurements on sample ZnO L with varying step widths. The scatter shows the measured Zn K fluorescence intensities in cps with the calculated depth profiles as solid lines. The quantified concentration values are plotted to the right y-axis, with dashed as certified and solid as calculated concentrations. The different step widths are displayed with an offset for clarity. Visually the reduction of the resolution is clearly discernible. Nonetheless the quantification works similarly well for all resolutions.

E. Bulk quantification results

Table S4: Listed are the calculated and certified concentrations and their deviation for the measured reference materials.

Element	Calculated concentration / %	Certified concentration / %	Deviation / %
BR A4			
Ca	0.5784 ± 0.0105	0.5932	- 2.5
Ba	1.62 ± 0.05	1.7913	- 9.4
Ti	3.03 ± 0.06	2.3377	29.6
Mn	14.9 ± 0.2	15.7215	- 5.2
Fe	0.829 ± 0.015	0.8113	2.2
Zn	5.67 ± 0.13	5.9451	- 4.6
Ge	0.072 ± 0.003	0.0555	28.9
Rb	0.115 ± 0.003	0.0366	214.3
BR B2			
Sn	0.77 ± 0.03	0.7246	5.7
Ca	15.8 ± 0.2	15.2231	3.7
Ti	0.790 ± 0.016	0.7193	9.9
Mn	0.852 ± 0.012	0.6893	23.5
Fe	8.67 ± 0.14	8.5331	1.6
Co	1.37 ± 0.03	1.2741	7.6
Ni	0.61 ± 0.02	0.6208	- 1.5
Cu	0.184 ± 0.004	0.1997	- 7.7
Ta	0.76 ± 0.03	0.6961	9.1
W	1.30 ± 0.03	1.4670	- 11.6
Zn	0.367 ± 0.008	0.3615	1.5
BR C3			
K	5.463 ± 0.109	5.7280	- 4.6
Ba	0.870 ± 0.017	0.8957	- 2.8
Ti	0.093 ± 0.004	0.0599	54.5
Pr	0.188 ± 0.007	0.1709	9.8
Nd	0.372 ± 0.005	0.3451	7.7
Mn	0.392 ± 0.006	0.3640	7.5
Fe	3.68 ± 0.06	3.7769	- 2.7
Ni	0.2204 ± 0.0106	0.2279	- 3.3
W	0.670 ± 0.012	0.7137	- 6.2
Ge	0.256 ± 0.012	0.1874	36.6
As	0.725 ± 0.013	0.5908	22.7
Bi	0.478 ± 0.011	0.4485	6.5
Ge	0.195 ± 0.004	0.1874	4.1

Element	Calculated concentration / %	Certified concentration / %	Deviation / %
BR D3			
Ca	11.1 ± 0.7	10.2202	8.8
La	0.84 ± 0.02	0.7504	12.1
Ce	0.697 ± 0.019	0.7172	- 2.8
V	0.54 ± 0.02	0.4817	12.8
Fe	0.46 ± 0.02	0.4057	11.6
W	0.264 ± 0.008	0.2538	3.9
Zn	2.97 ± 0.06	2.9726	- 0.1
Ga	0.364 ± 0.008	0.3422	6.4
Ge	0.298 ± 0.007	0.2846	4.7
As	1.61 ± 0.04	1.4087	14.6
BR E3			
K	0.777 ± 0.019	0.7886	- 1.5
Ba	4.71 ± 0.11	4.1200	14.3
La	0.298 ± 0.012	0.3411	- 12.7
Cr	0.465 ± 0.009	0.3832	21.3
Mn	5.58 ± 0.09	5.0340	10.8
Co	0.6270 ± 0.0112	0.5820	7.7
Ni	1.40 ± 0.03	1.4537	- 3.9
Cu	0.694 ± 0.018	0.6551	5.9
Zn	0.84 ± 0.02	0.7391	13.6
As	0.567 ± 0.012	0.3333	70.0
Sr	0.665 ± 0.014	0.2621	153.8
BR F3			
K	13.5 ± 0.4	15.1917	- 11.0
Ca	2.08 ± 0.05	2.0298	2.5
V	1.34 ± 0.02	0.9523	40.7
Sm	0.191 ± 0.004	0.1552	22.8
Fe	0.078 ± 0.002	0.0490	58.8
Co	0.217 ± 0.004	0.1966	10.1
Cu	1.45 ± 0.03	1.4380	0.8
Ta	0.40 ± 0.01	0.2948	37.3
Ga	0.0712 ± 0.0018	0.0670	6.2
BCR 129 Hay Powder			
K	2.81 ± 0.03	3.3800	- 16.8
Ca	0.70 ± 0.04	0.6400	8.7
Mn	0.0138 ± 0.0006	0.0072	92.0
BCR 176R Fly Ash			
Mn	0.054 ± 0.002	0.0730	- 26.6
Fe	0.516 ± 0.015	1.3100	- 60.6
Ni	0.0137 ± 0.0009	0.0117	16.7
Cu	0.141 ± 0.005	0.1050	34.4
Zn	1.44 ± 0.04	1.6800	- 14.5
Pb	0.490 ± 0.014	0.5000	- 2.0
BCR 667 Estuarine Sediment			
Cr	0.0117 ± 0.0005	0.0178	- 34.0
Mn	0.0786 ± 0.0015	0.0920	- 14.5
Fe	4.6 ± 0.1	4.4800	2.1
Ni	0.0148 ± 0.0006	0.0128	16.0
Cu	0.0152 ± 0.0004	0.0060	154.0
CC 144 Sewage Sludge			
K	0.223 ± 0.006	0.2900	- 23.0
Ca	2.82 ± 0.04	3.1000	- 9.0
Ti	0.176 ± 0.003	0.1500	17.5
Cr	0.0234 ± 0.0012	0.0168	39.6
Mn	0.0248 ± 0.0009	0.0352	- 29.6
Fe	2.45 ± 0.04	3.2900	- 25.7
Ni	0.0155 ± 0.0007	0.0091	70.8
Cu	0.0330 ± 0.0006	0.0348	- 5.3

Element	Calculated concentration / %	Certified concentration / %	Deviation / %
NIST 697 Bauxite			
Cr	0.098 ± 0.004	0.0684	43.1
Mn	0.42 ± 0.02	0.3175	33.4
Fe	16.9 ± 0.4	13.9887	20.9
NIST 1577 Bovine Liver			
Fe	0.0418 ± 0.0006	0.0268	55.9
Cu	0.0223 ± 0.0009	0.0193	15.4
NIST 1646 Estuarine Sediment			
Mn	0.074 ± 0.003	0.0375	96.0
Fe	4.02 ± 0.12	3.5000	14.9

Video S1: The video shows a full rotation of the 3D quantified positions of the goethite (red) embedded in smoky quartz (blue) analogue to figure 7 in the main text.